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ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

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STUDIES ON THE DIAGNOSTIC VALUE OF MODERN SEROLOGICAL TESTS FOR TUBERCULOSIS

BY

HELGE HONKAPOHJA

VOL. 32 **1954** **SUPPLEMENTUM 3**

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HELSINKI, FINLAND

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PREFACE

The incentive to carry out the investigation presented in this report originated when, in collaboration with Mr. V. Tommila, M. D., I studied the possibility of using the »cord factor», isolated by H. Bloch, as the antigen in a serological test for tuberculosis by the complement fixation technique. Professor E. Uroma, M. D., chief of the State Serum Institute, suggested in this connection the parallel use, as in the serological diagnosis of syphilis, of a number of serological tests for tuberculosis. Despite the great limitations of space and the heavy pressure of work at the Institute headed by Professor Uroma, I was permitted to conduct the studies there and I am greatly indebted to him for the unfailing interest with which he followed the progress of the work and for the support and guidance which made its continuation and completion possible.

Encouraging interest was shown in my investigation by Professor H. G. Haahti, M. D., Professor of Tuberculosis in the University of Helsinki, and his death prior to the completion of these studies was a great loss to me.

I am also deeply grateful to Professor K. O. Renkonen, M. D., chief of the Department of Serology and Bacteriology in the University of Helsinki, for his encouragement and practical advice at all times and for examining the completed report.

Docent J. Pätiälä, M.D., has been a valuable support to me in the handling of the clinical aspects of the case series, especially by instructing me in the classification and evaluation of the tuberculous cases.

I wish to express my thanks to Miss Eeva Alameri, Ch. E., and to Mr. A. Louhivuori, Mag. Phil., for friendly advice and assistance in the preparation and analysis of the antigen used in the tests. My thanks are also due to Miss Raija Ijäs and Mrs. Anna-Liisa Sallinen for willing help in the practical performance

of the tests. The assistance of Mr. M. Kajamaa, Mag. Phil., in the statistical treatment of the results is acknowledged with thanks.

I also extend my thanks to all persons who kindly have assisted me in the collection of blood samples and the selection of hospital records.

Finally I thank Miss Elvi Kaukokallio for translating this report into English.

Helsinki, April, 1954.

Helge Honkapohja.

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INTRODUCTION

Although tuberculosis, especially of the pulmonary type, can usually be readily recognised in x-ray and sputum examinations, attempts have been made for over 50 years to develop also a suitable serological test for tuberculosis. Among such tests are the agglutination reactions, various precipitation reactions, complement fixation reactions, and different chemical reactions, the last mentioned being usually based on changes in the blood proteins. The agglutination tests were soon discarded, as they proved to be highly non-specific and as the production of a homogeneous tubercle bacillus suspension was difficult. Among precipitation tests probably the best known are that of Lehmann-Facius and Loeschke, of 1926, in which an alcoholic extract of tuberculous tissue is used as antigen, and the Meinicke reaction of 1933, in which the antigen consists of structural parts of the tubercle bacillus obtained by extraction of bacilli with alcohol and water. The complement fixation tests most widely in use are those of Besredka, 1913, Boquet-Nègre, 1921, and Witebsky-Klingenstein-Kuhn, 1931. The Vernes resorcin flocculation test of 1926 is probably the most commonly used chemical reaction.

A new type of serological reaction was developed by Koegh, North and Warburton in 1947 (121). Their technique consists in adsorption of bacterial polysaccharides to the surface of red cells and agglutination of the treated cells by serum immunised with the bacterium corresponding to the polysaccharide used. In 1948 Middlebrook and Dubos (132) adapted this reaction to the study of tuberculosis and in 1950 Middlebrook (133) described a technique in which addition of complement produced hemolysis of the red cells by the serum of patients with tuberculosis.

Although research work on the hemagglutination and hemolysis reactions has been relatively active in recent years, there are

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very few comparative studies of these reactions and earlier serological tests for tuberculosis. Gernez-Rieux and Tacquet (104) compared the hemagglutination test with the Besredka and Boquet-Nègre tests in 124 cases of pulmonary tuberculosis and found that 85 per cent of the cases were positive in the hemagglutination test, 74 per cent in the Besredka test and 73 per cent in the Boquet-Nègre test. The control series comprised 61 sera of healthy persons, 5 per cent of which gave a positive reaction in the hemagglutination test, 23 per cent in the Besredka and 26 per cent in the Boquet-Nègre test. Lucentini and Boisvert (125) studied the same reactions as well as Middlebrook's hemolytic modification. In the latter, however, they used human O Rh — cells, which are poorly suited for this test. The incidences of positive reactions in the tuberculotic cases were as follows: hemagglutination test, 75 per cent; hemolytic modification, 45 per cent; Besredka test, 56 per cent; Boquet-Nègre test, 70 per cent. Popp (136), in a series of 140 cases, obtained definitely positive reactions in 66 cases in both the hemagglutination and the hemolysis tests. When using the Witebsky-Klingenstein-Kuhn and the Meinicke tests, 34 of these 66 cases were positive in the former but only 6 in the latter test. The results obtained in these comparative studies lead us to strongly believe that the new reactions offer improved possibilities for the employment of serological tests as a diagnostic aid in tuberculosis.

A fair amount of study has been devoted also in Finland to serological tests for tuberculosis. In 1936 Salmenkallio (165) published his investigation on the Witebsky-Klingenstein-Kuhn reaction, in 1939 Streng (76) employed the Witebsky-Klingenstein-Kuhn and the Meinicke tests in studying the blood sera and synovial fluid of patients with affections of the knee joint, and Honkanen (164) reported in 1940 the results of parallel tests with the Besredka and the Witebsky-Klingenstein-Kuhn reactions.

REWIEW OF THE LITERATURE

EARLIER INVESTIGATIONS WITH THE MEINICKE TEST FOR TUBERCULOSIS

Principle of the Test. — Meinicke based his test on the use of a compound antigen. For this purpose he prepared from tubercle bacilli an alcoholic extract containing lipids and an aqueous extract containing proteins and polysaccharides. Addition of these antigens in given proportions to the serum of a tuberculotic patient produced a precipitate, the nature of which was estimated in order to determine the positivity of the reaction.

Preparation of the Antigens and Development of the Technique. — Before Meinicke (51) published in 1933 the test he had developed especially for tuberculosis, Haag and Dane (26, 27) and Nagell (63) used a kind of modification of the Witebsky-Klingenstein-Kuhn reaction, in which they added to the antigen the immunity antigen originally developed by Meinicke in 1917. In his serological test of 1933 for tuberculosis Meinicke employed as antigen the alcoholic extract of tubercle bacilli, to which were added lipids from beef heart muscle and Witebsky-Klingenstein-Kuhn antigen. However, in the following year (52) he altered the composition of this antigen by substituting for the last mentioned component the water-soluble portion of tubercle bacilli. No exact description of the preparation of the antigens is given in the literature, but his technique was approximately as follows. The tubercle bacilli are first extracted with ether, which is discarded and the balance removed by desiccation. The bacterial mass is extracted first with alcohol and then with water, and balsam of tolu and Victoria blue are added in given proportions to the alcoholic extract to sensitise the reaction and facilitate its reading.

In his first reaction Meinicke used four test tubes for each serum. To the first tube he added strong alcoholic antigen, to the second

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weak alcoholic, to the third water-soluble, and to the fourth control antigen. For the last mentioned he used syphilis antigen M.K.R. II. In 1937 Meinicke (57, 58) changed his earlier test method by adding to the water-soluble antigens small amounts of beef broth in which the tubercle bacilli had grown, thus increasing the effect of the antigens. He altered the technique further by transfer to a three-tube system and by first using room temperature and then the water bath. The latter reading in particular he considered very important. In 1940 he published (60) the dried blood test, which he had developed for the diagnosis of tuberculosis and syphilis in mass examinations. In this test he employed only concentrated aqueous and alcoholic tuberculosis antigens.

Three different methods are used for reading the Meinicke reaction. In the *microscopic method* the test tube series are kept in the thermostat at +20° C for 2—3 hours and the reading is made at a 60-fold magnification. The development of a coarse, granular precipitate indicates a positive reaction and fine particles a negative reaction. In the *centrifugation method* the test tube series is centrifuged for 20 min. at a speed of 2000—2500 r.p.m., the supernatant fluid is discarded, and the test tubes are kept in an inverted position for 15 min. In a strong positive reaction the sediment will not flow at all, in a weak positive reaction it is partly fluid, and in a negative reaction it is completely fluid and runs along the tube wall. In the so-called *»Kuppen» technique* the test tube series is placed in the water bath for 24 hrs., after which a reading is taken and the tubes are shaken and allowed to stand at room temperature for 24 hrs. If the reaction is positive a granular precipitate covers the entire bottom of the tube; if it is negative a uniform fine precipitate is seen in the centre of the tube bottom.

The technique of the Meinicke reaction has not been modified to any great extent by other workers, who either have employed one of his methods as such or have used all his methods simultaneously or successively. Hartwich (30) added potassium or calcium chloride to the saline solution but this rendered reading of the control tube more difficult. Berdel and Bühler (3) observed that lamp heat in the micro method accelerated the reaction so that a reading was possible already after 45 min. Originally in the »Kuppen» technique the test series was first placed in the water bath at +37° C and then kept at room temperature, but, as already stated above,

Meinicke (57) reversed the order, as he considered that heat, if used first, rendered the reaction exceedingly weak. Reading of the reaction in the micro method is difficult and for this reason it has been abandoned later by many investigators, *e.g.*, Böhne (12), Dissman und Dissman-Wosak (17), Schulte-Tigges (70) and Jochims (35). Most workers regard the »Kuppen» technique as the best, and it has gained the widest use. Meinicke stated in 1943 (60) that he was inclined to prefer the centrifugation technique. Reading of results in the Meinicke test is generally considered relatively difficult, as stated for instance by Volk (83) and Böhne. Many workers, *e.g.*, Weiland (80) and Volk, used only the second reading, since the differentiation of positive and negative reactions is then less difficult.

Schulte-Tigges (70) considered Meinicke's dried blood reaction a good test to supplement x-ray examination, but Meinicke himself felt that it was not very suitable for mass examination.

POSITIVITY OF MEINICKE TEST IN TUBERCULOSIS

Meinicke (54) found that his test gave in pulmonary tuberculosis the strongest positive reaction in the chronic type with a tendency to scar formation, whereas in acute processes it was frequently negative. Böhne (12) made the same observation. Gaethgens and Schulten (22) found the test negative in tuberculous meningitis and miliary tuberculosis, and Kriech (43) obtained negative reactions in fresh infiltrations and miliary tuberculosis and in fatal cases of pulmonary tuberculosis. In the studies by Leitner (45) the reaction was negative in most cases of exudative pleuritis. Becker (2) stated that the Meinicke test gave the strongest positive reactions in chronic cases and weakly positive reactions in fresh cases of pulmonary tuberculosis and in mild processes in the apical and upper fields, whereas it was negative in the presence of extensive exudative changes or a small apical process in the lungs and in pneumothorax and miliary tuberculosis.

In childhood tuberculosis Kielhorn (38) obtained a positive Meinicke reaction in 90 per cent of cases and Zeyland and Piasecka-Zeyland (86) in 50 per cent only. Brackman (10) observed that the fresher the process and the younger the child, the more rarely the

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reaction was positive. Nordenfelt (64) reached the conclusion that the Meinicke test is of no clinical significance in childhood tuberculosis.

Comparative good results were obtained by Böhne (12) in surgical tuberculosis, for in his series of 308 cases the reaction was positive in 196 cases, or in 64 per cent. On the other hand, a positive reaction was obtained by Fernandez (20) in only 17 cases in a series of 40 cases. Gullotta (24) reported a positive reaction in 80 per cent of his cases of surgical tuberculosis. Gundel and Heine (25) used the Meinicke test in urogenital tuberculosis and found a positive reaction in 32 out of 50 cases. Krelle (42) generally obtained a weak positive reaction in surgical tuberculosis. In Streng's (76) series of 87 cases of bone tuberculosis 21 cases gave a definitely positive Meinicke reaction. Wulff (84) made the observation that the reaction usually was negative if the tuberculous process was in the small joints. Volk (83) did not ascribe great significance to this test in the diagnosis of bone tuberculosis, since it was negative in the initial stage and only became positive when the diagnosis could already be made by other means.

In tuberculosis of the skin the Meinicke test generally gives a negative or a weak positive reaction (4). In 100 cases of lupus Meinicke (56) found a positive reaction in 21 cases only. Laue (44) was unable to obtain any positive reactions in 35 cases of tuberculosis of the skin. According to Hassko and Müller (31) the reaction is rarely positive in this disease and they considered the process too small and too superficial to incite the formation of antibody in the blood.

In tuberculosis of the eye, Jacob (34) obtained a positive reaction in 33.4 per cent of his cases.

SO-CALLED NON-SPECIFIC REACTIONS

Meinicke (54) observed in his test a positive reaction as frequently in syphilis as in tuberculosis. In such cases a strong positive reaction was also seen in the control tube. To remove this drawback he developed a method of saturation in which 1.25 ccm of control antigen in the usual dilution was added to 0.5 ccm of serum, the mixture centrifuged for 15 min., the supernatant fluid poured into a test tube, and the result read in the usual manner. The satura-

tion could be carried out a number of times. Regardless of this treatment a part of the syphilitic sera remained positive. Müller and Schedtler (62) and Olinescu and Huhulea (66) were of the opinion that differentiation between syphilis and tuberculosis is not possible in the Meinicke test. On the other hand Meyer (61) and Waldvogel (78) considered differentiation possible by the saturation method in about 90 per cent of cases. Leitner (47) suggested heating of the serum to +62 — +64°C., since the syphilis antibodies are more sensitive to heat, or precipitation of the euglobulins since the syphilis antibodies accompany this fraction, whereas the tuberculosis antibodies follow the pseudoglobulin and albumin fractions.

Ohmichi (65) in examining 120 patients with leprosy found the Meinicke test positive in 78 per cent of the cases. It was positive in 6 cases in a series of 22 cases of typhoid fever studied by Böhm and Grüner (13). Kielhorn (38) observed that acute infectious diseases frequently gave a positive reaction. Meinicke (59) and Eickhoff (19) were of the opinion that infectious diseases other than tuberculosis may re-activate antibody formation against this disease. Spinedi (75) observed that patients with tetanus who had received large amounts of tetanus serum as a therapeutic measure frequently gave a positive reaction. Zeyland and Piasecka-Zeyland (86) obtained a positive reaction in 3 out of 6 cases of diphtheria, and all 5 diphtheria sera in Brackman's series (10) were positive. The positive reactions seen in leprosy and diphtheria were ascribed by Meinicke (59) and Eickhoff (19) to the relationship of the bacterial agents in these diseases to the tubercle bacillus, which is close enough to render the structure of their antigens similar. In diabetes, Kalk and Burgmann (37) frequently obtained positive reactions, whereas Schriek (72), in a series of 30 diabetic patients, found a positive reaction in 2 cases only. In a closer follow-up examination these cases, furthermore, proved to be tuberculotic. Non-specific reactions were seen by Böhne (12) in recent cases of endocarditis, in renal diseases with a high non-protein nitrogen level in the blood, and in cirrhosis of the liver associated with icterus. A positive reaction was frequently obtained by Eickhoff (19) in asthma and malaria. Corbia (15) and Besta (6) often found the test positive in rheumatoid arthritis, and Leitner (47) and Heine (32) in pregnancy.

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COMPARISON OF THE MEINICKE TEST AND OTHER SEROLOGICAL TESTS FOR TUBERCULOSIS

Böhm and Grüner (13) carried out parallel tests with tuberculotic sera, using the Besredka, Boquet-Nègre, Kabelik-Zdaril, Witebsky-Klingenstein-Kuhn and Meinicke reactions. In their study the Meinicke and Besredka tests proved the best and gave positive reactions in over 70 per cent of cases of pulmonary tuberculosis. However, the control series gave a positive Besredka reaction in 31.3 per cent of cases but a positive Meinicke reaction in 6 per cent only. Heymer and Schulte-Tigges (33) made comparative studies of the Besredka, Lehmann-Facius and Loeschke, and Witebsky-Klingenstein-Kuhn tests and of three different techniques of the Meinicke test. The best proved to be Meinicke's »Kuppen» test, which in active pulmonary tuberculosis was positive in 75.4 per cent. Mazet (49) carried out parallel tests with the Besredka and Meinicke reactions, which gave approximately the same incidence of positive results in tuberculosis; however, the Besredka gave a larger number of non-specific reactions. These tests were also studied by Olinescu and Ghioghiu (67), who found that positive reactions were equally frequent in both tests in exudative tuberculosis but more frequent in the Meinicke test in fibrotic tuberculosis. The Meinicke »Kuppen» test and the Boquet-Nègre test were used by Zeyland and Piasecka-Zeyland (86), who obtained a larger number of positive reactions by the latter method. In tuberculosis of the bone Streng (76) obtained positive reactions in 14.4 per cent with the Witebsky-Klingenstein-Kuhn and in 24.1 per cent with the Meinicke test. Koss (40) used in parallel tests the Meinicke and the Witebsky-Klingenstein-Kuhn methods as well as the latter method sensitised with ammonium sulphate and found the Meinicke reaction to be best.

COMPARISON OF THE MEINICKE TEST AND SOME CLINICAL TESTS

Gaehtgens and Schulten (22) demonstrated that the results of the tuberculin and Meinicke tests do not conform, as healthy tuberculin-positive persons may give a negative Meinicke test, and a

negative tuberculin test may be seen in association with positive Meinicke reaction in tuberculosis. The same observation was made by Agostoni (1). Lämmli (48) stated that there apparently exists a marked difference between the tuberculin-sensitivity of the skin and the amount of antibody in the serum. Bernáth (5) studied the correlation between a positive Meinicke reaction and the intensity of the Mantoux skin test and found no conformity.

Croce (16) observed no correlation between the Meinicke test and the blood sedimentation rate, and he therefore considered the former a true immunity reaction and not merely a blood lability test. Agostoni, on the other hand, observed that a negative Meinicke reaction was frequently obtained in the presence of a low sedimentation rate. Hardt (29) usually found the Meinicke reaction positive when the blood lymphocyte count was high. Eickhoff (19) also considered the Meinicke test a true antigen-antibody reaction. This opinion he based on the negative reaction obtained in severe cases of pulmonary tuberculosis associated with high fever and a high sedimentation rate and, on the other hand, the strong positive Meinicke reaction that might be obtained even in the presence of a low sedimentation rate. Evidence in the same direction was the reversal of the positive blood lability reactions on slight dilution of the serum, whereas the Meinicke test frequently remained positive even if the serum was diluted 1: 20.

SUMMARY OF RESULTS OBTAINED WITH THE MEINICKE TEST FOR TUBERCULOSIS

To obtain a better review of the results reported by various investigators I have compiled in table 1 the results I have found in the literature. Most of the workers have listed the different forms of pulmonary tuberculosis separately, indicating a higher incidence of positive reactions in the fibrotic forms than in the exudative forms. In this table, however, I have differentiated only the active and the inactive forms of pulmonary tuberculosis. Where a special category of patients was concerned, this is stated in the »Remarks» column.

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TABLE 1

MEINICKE TEST RESULTS OBTAINED BY VARIOUS AUTHORS

Author and Year	Total No. of Cases	Active Pulmonary Tuberculosis	No. of Positive Reactions	% of Positive Reactions	Inactive Pulmonary Tuberculosis			Extrapulmonary Tuberculosis			Non-Tuberculous Diseases			Healthy Subjects			Remarks
						No. of Positive Reactions	% of Positive Reactions		No. of Positive Reactions	% of Positive Reactions		No. of Positive Reactions	% of Positive Reactions		No. of Positive Reactions	% of Positive Reactions	
1934																	
Domingo and Sayé	105	105	95	87	—	—	—	—	—	—	—	—	—	—	—	—	—
1935																	
Berdel and Bühler	?	?	?	96	?	?	20	—	—	—	—	—	—	—	—	—	—
Beuing	204	160	146	91	—	—	—	4	1	25	4	1	25	36	1	35	—
Böhne	3699	1156	920	80	—	—	—	308	196	64	2235	25	1	—	—	—	—
Böhm and Grüner	?	?	?	76	—	—	—	—	—	—	?	?	6	—	—	—	—
Croce	130	59	55	93	51	14	27	—	—	—	20	0	0	—	—	—	—
Dissman and Dissman-Wosak	262	214	136	64	—	—	—	—	—	—	—	—	—	48	14	30	—
Fernandez	60	—	—	—	—	—	—	40	17	40	20	1	5	—	—	—	—
Hartwich	1647	1400*	800*	57	—	—	—	47	7	16	200	8	4	—	—	—	*No exact figures Children
Jochims	116	50	13	26	66	2	3	—	—	—	—	—	—	—	—	—	—
Kalk	84	39	33	85	—	—	—	—	—	—	45	11	24	—	—	—	—
Müller and Schedtler	316	145	105	72	—	—	—	64	26	41	74	17	24	33	3	9	—
Spinedi	321	105	93	89	—	—	—	—	—	—	216	26	12	—	—	—	—
Viegener	400	400	320	80	—	—	—	—	—	—	—	—	—	—	—	—	—
Zech	505	239	195	81	—	—	—	26	11	42	240	20	8	—	—	—	—
1936																	

1936																	
Boas and Freudenthal	1276	721	633	88	34	25	74	—	—	—	—	—	—	521	3	0.6	—
Brackman.....	411	334	192	58	19	16	85	23	10	43	35	12	33	—	—	—	—
Böhne	10211	1825	1359	74	285	10	4	—	—	—	5991	113	2	2110	0	0	—
Gullotta	50	—	—	—	—	—	—	34	27	80	16	13	81	—	—	—	—
Hardt	87	87	84	97	—	—	—	—	—	—	—	—	—	—	—	—	—
Heymer and Schulte-Tigges	138	138	104	75	—	—	—	—	—	—	—	—	—	—	—	—	—
Kriech	425	175	153	86	—	—	—	—	—	—	250	9	4	—	—	—	—
Lämmli	234	179	134	75	—	—	—	25	15	60	18	0	0	12	0	0	—
Mazet	207	40	23	58	—	—	—	—	—	—	167	5	3	—	—	—	—
Olinescu and Huhulea	474	203	138	68	—	—	—	—	—	—	271	100	37	—	—	—	—
Schulte-Tigges	936	886	727	82	?	?	50	—	—	—	?	?	40	50	5	10	—
Trautwein.....	?	?	?	74	—	—	—	—	—	—	—	—	—	—	—	—	—
Verdina	482	185	159	86	139	30	21	—	—	—	148	12	8	10	0	0	—
Zeyland and Piasecka-Zeyland	479	282	242	86	—	—	—	7	6	86	108	39	36	82	43	52	—
Wawrzyniec	231	170	133	78	—	—	—	—	—	—	61	11	18	—	—	—	—
1937																	
Bortolozzi	277	177	152	86	—	—	—	—	—	—	50	3	6	50	0	0	—
Fröhlich	202	168	99	59	—	—	—	—	—	—	34	6	18	—	—	—	—
Goralewski and Boedicker ..	235	59	?	?	13	?	?	81	59	73	—	—	—	82	3	3	—
Leitner	316	228	182	80	33	10	33	24	16	67	—	—	—	31	6	19	—
Nordenfelt	100	47	7	15	7	1	14	—	—	—	46	5	11	—	—	—	Children
Olinescu and Ghiorghiu	500	221	139	63	—	—	—	—	—	—	279	98	35	—	—	—	—
Pieck	675	433	329	76	134	25	19	—	—	—	108	16	15	—	—	—	—
Spies	66	66	35	53	—	—	—	—	—	—	—	—	—	—	—	—	—
Waldvogel	284	27	25	93	—	—	—	—	—	—	41	21	51	216	13	6	—
1938																	
Bernáth	?	?	?	63	—	—	—	—	—	—	—	—	—	—	—	—	—
Hassko and Müller	329	143	113	80	—	—	—	66	17	26	—	—	—	120	8	7	—
Laue	25	25	23	90	—	—	—	—	—	—	—	—	—	—	—	—	—
Leitner	241	191	166	87	13	4	31	17	14	82	7	2	29	13	2	15	—
Spies	252	252	102	41	—	—	—	—	—	—	—	—	—	—	—	—	—

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cont.

Author and Year	Total No. of Cases	Active Pulmonary Tuberculosis	No. of Positive Reactions	% of Positive Reactions	Inactive Pulmonary Tuberculosis	No. of Positive Reactions	% of Positive Reactions	Extrapulmonary Tuberculosis	No. of Positive Reactions	% of Positive Reactions	Non-Tuberculous Diseases	No. of Positive Reactions	% of Positive Reactions	Healthy Subjects	No. of Positive Reactions	% of Positive Reactions	Remarks
1939																	
Corbia	216	216	133	62	—	—	—	—	—	—	—	—	—	—	—	—	—
Gundel and Heine	130	—	—	—	—	—	—	50	32	64	80	11	14	—	—	—	—
Jacob	?	?	?	84	—	—	—	?	?	33*	—	—	—	—	—	—	*tb of eye
Streng	201	—	—	—	—	—	—	87	21	24	114	3	4	—	—	—	—
1940																	
Carinci and Re	?	?	?	60	?	?	40	—	—	—	?	?	25	?	?	16	—
Wulff	39	—	—	—	—	—	—	39	26	67	?	?	8	—	—	—	—
1941																	
Becker	663	663	533	80	—	—	—	—	—	—	—	—	—	—	—	—	—
Koulumies	278	168	129	77	—	—	—	—	—	—	—	—	—	110	1	1	—
Leitner	296	?	?	86	87	28	32	122	95	78	22	6	27	65	12	18	—
Mc Menemey	772	383	300	78	5	4	80	52	17	33	332	62	19	—	—	—	—
Volk	30	13	11	85	17	?	?	—	—	—	—	—	—	—	—	—	—
1944																	
Heine	1878	394	355	90	—	—	—	782	436	56	702	29	4	—	—	—	—
1949																	
Koss	110	102	77	75	—	—	—	8	7	88	—	—	—	—	—	—	—
Total	31605	13273	9902	74.6	903	169	18.7	1906	1056	55.4	11934	685	5.7	3589	114	3.2	

THE MIDDLEBROOK-DUBOS HEMAGGLUTINATION TEST AND STUDIES ON THIS REACTION

Principle of the Test. — When polysaccharides fractionated from bacteria are precipitated from the solution to the surface of red cells, these cells agglutinate when added to the corresponding immune serum. This reaction is therefore more properly a precipitation reaction, in which the sensitised red cells are the precipitate that indicates the intensity of reaction.

Technique. — The serum to be tested is diluted with physiological saline solution 1: 1 and inactivated in the water bath at +56° C for ½ hr. The treated serum is absorbed into sheep red cells by adding 0.2 ccm of sheep cells to 2 ccm of serum dilution. The suspension is allowed to stand for 20 min. at room temperature and the tube is shaken from time to time. The cells are centrifuged to the bottom and the serum is re-treated once more in the same manner. Before sensitisation the red cells are washed three times with a 6-fold volume of physiological saline solution and centrifuged at 2000 r.p.m. for 20 min. between washings. The washed cells are stored at +4° C. However, even at this temperature they will be serviceable for only 3 days. For sensitisation, 0.5 ccm of washed sheep red cells are suspended in 10 ccm of extract of tubercle bacilli, prepared as will be described later. The suspension is placed in the water bath at +37° C for 2 hrs. and is shaken frequently. The cells are then centrifuged and the fluid is discarded. The cells are washed three times and finally suspended in 100 ccm of physiological saline solution. This will yield a 0.5 per cent suspension of sensitised red cells for use in the test.

Preparation of the Antigen. — For sensitisation of the red cells Middlebrook and Dubos prepared an antigen from strain H_{37} Rv, which had been grown on fluid medium for 14 days. The bacillary mass was collected by filtration, washed in distilled water and then with acetone, and 4 gm of this mass was added to 100 ccm of 88 per cent phenol and mixed. After 20 hrs., during which the suspension was shaken several times, the supernatant brownish fluid was discarded. This procedure was repeated and the precipitate was washed in 90 ccm of 0° C acetone to remove the phenol. Of this bacillary mass 0.5 gm was added to 65 ccm of phosphate-buffered saline and methanol, shaken during 20 hrs. and then centrifuged.

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The fluid was dialysed against distilled water for 2—3 days, concentrated by evaporation to 20 ccm, any turbidity that had developed was removed by centrifugation, the pH was adjusted with NaOH to 6.5—7.0, and the solution was rendered isotonic with a saturated solution of NaCl. Sensitisation of 0.5 ccm of sheep red cells was carried out with 10 cm of this antigen solution.

Middlebrook and Dubos (132) observed that Gilliland Old Tuberculin (Wyeth) could also be utilised for the sensitisation of red cells. This observation was followed by numerous experiments on the use of tuberculin for this purpose. Lederle 4 times concentrated tuberculin in dilutions 1: 12—1: 15 has gained the widest use, having been employed by, *e.g.* Scott and Smith (142), Rothbard *et al.* (138), Hall and Manion (113), Kirby *et al.* (120), Maillard and Gagliardo (128), Thalhimer and Rowe (149), Bunn *et al.* (94), Colwell and Pitner (97), Gerstl *et al.* (109), Haahti and Raitio (112), Hentel and Guilbert (115), Madsen and Oeding (127) and Mollov and Kott (135).

Other tuberculins used for this purpose are the following:

Koch's Old Tuberculin, in 1 per cent concentration: Gernez-Rieux and Tacquet (106); diluted 1: 80 and deglycerinated: Avezzù (89)

Tuberculin G. T. Hoechst, in dilution 1: 15: Brodhage (93), Hein and Sroka (114),

Sauton Tuberculin »Berna» from the human and the bovine strain, deglycerinated with alcohol, 1 ccm (300,000 units) used for sensitisation of 0.15 ccm sheep cells: Brodhage (93), Weidmann (155),

Old Tuberculin Burroughs Wellcome, in dilution 1: 5: Hilson and Elek (116), Sherris (143),

Danish Old Tuberculin, in 1.7 concentration, diluted 1: 25: Koulumies and Haahti (122),

Norwegian Veterinary Institute's tuberculin, deglycerinated with alcohol: Madsen (126),

Dialysed Evans Tuberculin: Sherris (143),

Denken Tuberkulin, in dilution 1: 10: Yaoi *et al.* (161),

Purified protein derivatives of tuberculin, in concentrations of 0.2—2.5 mg/ccm, such as PPD Parke Davis: Hilson and Elek (116); PPD Copenhagen: Brodhage (93), Popp (136); PPD Weybridge: Boyden (92), Sherris (143).

Pasteur Institute's precipitated tuberculin, in dilution 1: 100: Gernez-Rieux and Tacquet (105), Sohier *et al.* (146).

The Pasteur Institute later prepared a special tuberculin for serological tests, called I.P. 48. There are available a protein-rich preparation I.P. 48 Pt. containing 8.5—9.5 per cent of nitrogen, and a polysaccharide-rich I.P. 48 St., containing only 0.42 per cent of nitrogen and polysaccharide in the amount of 0.14 mg/ccm. This tuberculin has been used as antigen by Gernez-Rieux and Tacquet (107), Sohier *et al.* (147), Hein and Sroka (114), Lucentini and Boisvert (125) and Ulstrup (154).

Gernez-Rieux and Tacquet (108) carried out experiments with two rabbit sera, one of which was immunised with BCG strain, to determine the amount of various tuberculins required to sensitise 0.1 ccm of sheep cells to obtain a hemagglutination titre of 1: 16 for immunised rabbit serum. They found that the following amounts should be utilised:

I.P. 48 Pt.	400 units
I.P. 48 St.	400 »
PPD Copenhagen	2,000 »
Tuberculin G. T. Hoechst.....	20,000 »
Sauton Tuberculin »Berna» from human strain	1,600 »
» » » » bovine »	20,000 »
PPD Weybridge	0.5 mg
Lederle Tuberculin, 4 × concentrated, 1: 12	1 ccm

It is to be noted that the Hoechst tuberculin also produced agglutination in normal rabbit serum. Seibert's purified PPD gave no agglutination.

HEMAGGLUTINATION INHIBITION TEST

Middlebrook and Dubos (132) developed a method based on the inhibition of hemagglutination of red cells. They selected a serum with a high titre and diluted it to slightly below the maximum agglutination titre. The antigen to be examined was added to the serum in a decreasing series of dilutions. The tubes were placed in the water bath at +37° C for 45 min. and a 0.5 per cent suspension of sensitised cells was added to each test tube. The usual procedure for the hemagglutination test was then followed. In this

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manner it was possible to determine the limit of inhibition of hemagglutination by the antigen. In producing immune sera Fenner and Leach (98) employed for immunisation pathogenic and apathogenic strains of *Mycobacterium* and then absorbed the sera with corresponding and other strains. In cross tests the human and bovine strains removed the antibodies completely. In the same test it was observed that *Mycobacterium ulcerans* is more closely related to the species of *Mycobacterium* that cause mammalian tuberculosis than to apathogenic species. Gaby *et al.* (101, 102) prepared tuberculins from various strains of *Mycobacterium* and used them for the absorption of immune sera. It was observed that all the antibodies in the serum were not always removed and that some antibody remained which corresponded to the tuberculin from another strain. In general, tuberculins prepared from pathogenic strains were better able to remove antibody than those from apathogenic strains. Gaby *et al.* isolated strain No. 350, whose tuberculin removed the hemagglutinins from the sera of non-tuberculous subjects and from the sera of three-fourths of the persons inoculated with BCG vaccine, but not from the sera of tuberculotics. They considered it possible to compare the potency of various tuberculins by means of their agglutinin-removing capacity, whereas Boulanger and Konst (91) regarded this method less reliable than the skin test. Madsen (126) demonstrated that already a very small amount of the red cell sensitising factor was capable of inhibiting hemagglutination in the serum of tuberculous patients. He also demonstrated that when tuberculin was absorbed by a large amount of red cells the sensitising factor was removed but the inhibitory factor remained.

OTHER STUDIES ON THE HEMAGGLUTINATION REACTION

Ghione (110) used tuberculin fractions of different kinds in examining the sera of 40 persons affected with tuberculosis. The best was the tuberculin extracted with phenol and insoluble in acetone. Hilson and Elek (116) prepared a special polysaccharide fraction which they called C.C.F. and with which they obtained better results than with Old Tuberculin Burroughs Wellcome. Iland (119), in studying different polysaccharide fractions previously

isolated from the tubercle bacillus, obtained positive results in precipitation tests with horse, rabbit and guinea pig immune sera, whereas the sera of tuberculous patients gave negative results. The hemagglutination and hemolysis reactions also were negative. He concluded that the human serum does not contain antibody corresponding to completely protein-free polysaccharide. Middlebrook (134) prepared from the tubercle bacillus a fraction containing only about 1 per cent of nitrogen and 90—98 per cent of polysaccharide. Only about 40 per cent of the polysaccharide was adsorbed into the red cells, the remaining portion not being adsorbed even into red cells treated with tannin. Pound (137) analysed the protein-polysaccharide fraction from the tubercle bacillus mass using 50, 61, 75, 87 and 92 per cent ethanol precipitation. He determined the amount of proteins, hexoses and pentoses in each fraction by the biuret, Molisch and Bial reactions. The fractions which were precipitated in 50, 61 and 87 per cent ethanol were protein-free. Fraction 3 (75 per cent) gave the best results in the hemagglutination test, whereas fractions 2 and 4 were better suited for the hemolysis test. The fraction obtained with 92 per cent ethanol contained a factor which caused non-specific agglutination up to titre 1: 32.

Colwell and Pitner (97) sensitised colloidal silver with tuberculin and compared the reaction with red cell agglutination. The titre in most cases was lower and the specificity was not improved. Gerstl *et al.* (109) made an effort to improve the sensitivity of the hemagglutination reaction by adding 5 per cent bovine albumin and glycine to buffered saline and by treating the red cells with Forsman's antibody and polyvinylpyrrolidine. After these procedures the titre was raised to two-fold at the most. Grabar *et al.* (111) preceded the sensitisation procedure by tannic acid treatment. In their opinion this treatment would absorb from the antigen into the red cells certain substances which react with the protein antibody in the blood; however, if normal cells are used, these substances react with the polysaccharide antibody in the blood. For this reason, when cells sensitised with polysaccharide are used the reaction may sometimes be negative in tuberculous patients, but if the same serum is tested with red cells pretreated with tannic acid and then sensitised with an antigen containing tuberculin protein a positive reaction and a very high titre may be obtained. Hein and Sroka (114), again, considered treatment with trypsin superior to tannin

treatment. Meynell (131) carried out the hemagglutination reaction by Coombs's direct test technique. For the sensitisation of human 0 Rh— cells he used as antigen 0.2 mg of PPD per ccm. One drop each of serum, of 2 per cent sensitised cells and of anti-human globulin were pipetted on a slide and the time when agglutination became visible to the naked eye was determined. Only 4 sera in a series of 20 tuberculotic bloods had a higher titre than that obtained by the usual hemagglutination technique. Thalhimer and Rowe (149) used a 15 per cent suspension of red cells in the slide method. In their opinion better results were obtained in this case than when the usual hemagglutination method is used. Yaoi *et al.* (161) utilised a 10—15 per cent suspension of sensitised cells and a serum dilution of 1: 2. etc. The number of positive reactions was the same as in the hemagglutination test but the number of doubtful reactions was greater. The same method was utilised by Witmer (156) in studying tuberculosis of the eye, but for serum he substituted active aqueous humour from the anterior chamber, using it in dilutions 1: 10, 1: 20, 1: 30, and so on.

THE MIDDLEBROOK HEMOLYSIS TEST

Principle of the Test. — The cells are sensitised in the same manner as described for the hemagglutination test. Addition of complement will cause hemolysis of the sensitised red cells in the serum of tuberculous subjects but not in that of healthy individuals. The test involves titration of the hemolysing capacity of the serum, whereas the amounts of complement and antigen remain constant.

Technique. — In this test, also, Middlebrook (133) employed a 0.5 per cent suspension of sensitised sheep red cells. Sensitisation was carried out as in the hemagglutination test. The complement was absorbed with sheep red cells by adding 1 ccm of sheep cells to 5 ccm of guinea pig serum. The suspension was kept at +4° C for 10 min., after which the cells were centrifuged. The complement will retain its activity for 3—4 days only. Since the sensitised cells are exceptionally fragile, an excess of complement may *per se* produce hemolysis; for this reason the complement should be accurately titrated. After determination of the unit amount of complement Middlebrook used in the test an amount three times this unit.

Before the final titre reading Middlebrook kept the test tube series at a temperature of +37° C for 1 hr., shaking the tubes at intervals of 15 min.

HEMOLYSIS INHIBITION TEST

The technique in this test is in principle the same as in the hemagglutination inhibition test.

Gernez-Rieux and Tacquet (108) used this method for parallel tests of I.P. 48, its polysaccharide fraction, precipitated Pasteur Institute tuberculin, 4 times concentrated Lederle tuberculin, Besredka and Boquet-Nègre antigens, extract of *Mycob. phlei*, extract of strain H_{37} Ra,BCG culture, and purified protein fraction of I.P. 48. Hemolysis was not inhibited by the Besredka and Boquet-Nègre antigens, bacillar extract of *Mycob. phlei* or purified protein fraction of I.P. 48. On the basis of this experiment they concluded that the sensitising substance in the hemolysis reaction is some kind of a polysaccharide.

ANIMAL EXPERIMENTS AND BCG VACCINATION

It was found by Gernez-Rieux and Tacquet (104) that normally the titre for the hemagglutination and hemolysis reactions in rabbit and guinea pig sera is 1: 2—1: 4. The killed bacilli and intradermal BCG vaccination caused a rise in the titre to 1: 64 within 15—30 days. The titre remained unchanged for about 200 days, after which a gradual decline commenced. After intravenous injection of live tubercle bacilli into animals the rise in the titre was considerably greater (96). Bunn *et al.* (94) observed that the titre was definitely increased in animals by infection with tuberculosis but only slightly by the intradermal tuberculin test. Brodhage (93) demonstrated that the hemagglutination titre in rabbits, even healthy ones, was 1: 160 when tuberculin prepared from human strains was employed to sensitise the red cells, but with bovine tuberculin it was low or entirely negative. This, in his opinion, accounted for the greater resistance of rabbits to infection by the human strain. Tsuchiya (151) injected guinea pigs with heat-killed tubercle bacilli. The tuberculin test became positive more slowly than the hemagglutination test in these animals. However, he

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TABLE

HEMAGGLUTINATION TEST RESULTS

Author and Year	Lowest Positive Titre	Tuberculosis		
		Total	+	—
1950				
Rothbard *et al.*	≥ 1: 8	168	155	13
Smith and Scott	≥ 1: 2	104	83	21
Sievers *et al.*	≥ 1: 16	86	46	40
Gernez-Rieux and Tacquet	≥ 1: 16	504	402	102
Sohier *et al.*	≥ 1: 9.2	30	20	10
1951				
Adcock *et al.*	≥ 1: 8	66	36	30
Anderson and Platou	≥ 1: 4	52	28	24
Avezzù and Morsica	≥ 1: 16	34	31	3
Brodhage	≥ 1: 10	104	97	7
Hall and Manion	≥ 1: 8	151	85	66
Kirby *et al.*	≥ 1: 8	63	33	30
Levine	≥ 1: 8	96	74	12
Thalhimer and Rowe	≥ 1: 16	75	43	32
Yaoi *et al.*	≥ 1: 10	155	144	11
Young and Leonard	≥ 1: 16	136	78	58
1952				
Gernex-Rieux and Tacquet	≥ 1: 16	660	397	263
Haahti and Raitio	≥ 1: 8	—	—	—
Hentel and Guilbert	≥ 1: 16	345	81	264
Koulumies and Haahti	≥ 1: 8	149	107	42
Lewin *et al.*	≥ 1: 8	93	62	31
Lucentini and Boisvert	≥ 1: 16	69	52	17
Madsen and Oeding	≥ 1: 16	119	75	44
Mollov and Kott	≥ 1: 8	100	69	31
Popp	≥ 1: 8	275	233	42
Rowe *et al.*	≥ 1: 8	32	26	6
Schwartz *et al.*	≥ 1: 32	331	70	261
Ulstrup	≥ 1: 8	222	180	42
Yaoi *et al.*	≥ 1: 10	45	26	19
1953				
Howard *et al.*	≥ 1: 8	172	114	56
Sherris	≥ 1: 8	49	32	17

2

OBTAINED BY VARIOUS AUTHORS

Non-Tuberculous Diseases			Healthy Subjects			Remarks
Total	+	—	Total	+	—	
106	0	106	110	0	110	
43	10	33	71*	36*	35*	*Tuberculin-negative cases
			28	2	26	
102	18	84	19	0	19	
70	3	67	244	17	227	
15	0	15	140	19	121	
—	—	—	201*	27*	174*	
			55	17	38	
—	—	—	107*	42*	65*	Children
			184	12	172	
—	—	—	—	—	—	Bone and urogenital tb
3	3	0	—	—	—	
—	—	—	61*	13*	48*	
			67	7	60	
—	—	—	251	24	227	
—	—	—	40	4	36	
—	—	—	31	6	25	
149	25	124	157	14	143	
—	—	—	411	52	359	
440	54	386	259	24	235	
—	—	—	105	6	99	Umbilical cord bloods
—	—	—	130	7	123	
196	41	155	198	70	128	
14	2	12	119	18	101	
16	2	14	12	2	10	
—	—	—	100*	15*	85*	
			240	41	199	
—	—	—	88	26	62	
154	11	143	62	1	61	
—	—	—	31	13	18	
50	2	48	297	21	276	
9	2	7	1405	343	1062	
59	4	55	—	—	—	Urogenital and skin tb
257	64	193	30*	7*	23*	
			50	12	38	
5	0	5	80	11	69	

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TABLE 3

HEMOLYSIS TEST RESULTS OBTAINED BY VARIOUS AUTHORS

Author and Year	Lowest Positive Titre	Tuberculosis			Non-Tuberculous Diseases			Healthy Subjects			Remarks
		Total	+	—	Total	+	—	Total	+	—	
1950											
Middlebrook	≥1: 8	27	24	3	10	0	10	—	—	—	
1951											
Gernex-Rieux and Tacquet	≥1: 16	208	158	50	121	16	105	—	—	—	
Hall and Manion	≥1: 32	151	81	70	—	—	—	61*	12*	49*	*Tuberculin-negative cases
								67	6	61	
Maillard and Gagliardo	not mentioned	63	59	4	121	6	115	158	0	158	
1952											
Colwell and Pitner	≥1: 16	21	18	3	13	3	10	—	—	—	
Lucentini and Boisvert	≥1: 16	67	30	37	13	3	10	12	1	11	
Gernex-Rieux and Tacquet	≥1: 16	191	144	47	225	23	202	172	21	151	
Maillard	not mentioned	136	126	10	181**	37	144	173	0	173	**Incl. 24 cases of leprosy, 23 of which +
Popp	≥1: 8	275	176	99	154	11	143	62	0	62	
Rowe *et al.*	≥1: 3	32	28	4	—	—	—	31	9	22	
Schwartz *et al.*	≥1: 32	331	69	262	50	2	48	297	23	274	
1953											
Sherris	≥1: 8	42	30	12	5	1	4	80	4	76	

observed no correlation between the intensity of the tuberculin test and the hemagglutination and hemolysis titres.

Gernez-Rieux and Tacquet (104) in experiments with 45 BCG-vaccinated persons found a hemagglutination titre of over 1: 16 in 33 persons 1 month after vaccination. A follow-up examination was possible in 16 cases only, and only 4 had a positive titre 2 months after vaccination. Similarly, Weidmann (155) observed that BCG vaccination increases the titre for a short time only. Ulstrup (153) found an increased titre in 3 BCG-vaccinated individuals but only one of them developed tuberculin positivity.

SUMMARY OF RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS

The results obtained by the various investigators in hemagglutination and hemolysis tests are compiled in tables 2 and 3. I have not added these results together in view of the variabilities in the antigens, positive titre limits and study material used by the different workers. Some series included a large number of inactive cases, whereas others consisted solely of active cases of pulmonary tuberculosis, which gave a greater number of positive reactions.

SUMMARY OF THE LITERATURE

The antigens used in the Meinicke test are in a way standardised by being prepared in one laboratory only. Similarly, Meinicke developed his test technique to a final form which has been hardly modified by any other worker. However, the positivity of the results seems to vary greatly with different investigators. This is apparently due, on the one hand, to variations in the series studied and, on the other hand, to the difficulty of evaluating positive reactions. However, the opinion appears to be generally accepted that chronic cases of pulmonary tuberculosis with a tendency to cicatrisation most frequently give a positive reaction, whereas acute forms, small apical processes and miliary tuberculosis give mostly a negative reaction.

In childhood tuberculosis, positive reactions are considerably fewer. Although the results obtained by different investigators vary greatly it seems even by the most optimistic estimation that only at

the most 50 per cent of cases of chilhood tuberculosis give a positive reaction in the Meinicke test. In surgical tuberculosis the incidence of positive reactions appears to be of the same magnitude, whereas in tuberculosis of the skin it seems to be the same as in non-tuberculous diseases.

Unfortunately the Meinicke test frequently gives a positive reaction in non-tuberculous diseases, as in syphilis and leprosy, in which the positive incidence is as high as in tuberculosis. According to reports in the literature, a non-specific positive reaction is obtained in acute infectious diseases, rheumatoid arthritis, malaria and pregnancy. Positive reactions are also produced by bacterial endocarditis, cirrhosis of the liver, kidney diseases and asthma. Using for comparison the final figures in summary (table 1), the Meinicke test may be regarded as quite satisfactory, for according to these data the reaction was positive in 74.6 per cent of cases of pulmonary tuberculosis in the active stage, in 18.7 per cent of cases of inactive pulmonary tuberculosis, in 55.4 per cent of cases of extrapulmonary tuberculosis, in 5.7 per cent of other diseases, and in 3.2 per cent of healthy individuals. The low incidence in the category of other diseases was to a great part due to the fact that Böhne's series of 11,934 cases included 8,226 cases which were collected chiefly from medical hospitals and therefore gave a lower incidence of positive reactions than normally is the case.

The experiments reported in the literature on the hemagglutination and hemolysis reactions have been centred around the following points:

a) development of the test technique,
b) study and purification of the antigens,
c) study of the clinical value of the tests.

Attempts have been made to improve the specificity of the reactions by substituting colloid particles for red cells in the sensitisation process. However, no improvement has been seen in the results. A more sensitive reaction has been sought by the addition of glycine, albumin and globulin, but also with meagre results. Improvement of the antigen has been attempted by preparing from the tubercle bacilli or tuberculins some fractions with a higher protein or polysaccharide content, but without noteworthy success. Completely purified protein and polysaccharide preparations have

proved inactive when used as antigens. So-called purified protein tuberculins have been active, it is true, but they have been found to contain 2—5 per cent of polysaccharides. It still remains to be explained whether the active substance in the reaction is a protein or a polysaccharide. The series studied have generally been too small to give conclusive evidence of the clinical usefulness of the hemagglutination and hemolysis tests. Furthermore, the reports in the literature are not comparable because of the marked variations in the antigens and titre limits. However, Gernez-Rieux and Tacquet (108), after experiments with the hemagglutination test, using 2,489 sera, and with the hemolysis test, using 845 sera, considered these tests very satisfactory.

THE PROBLEMS

As is seen from tables 2 and 3 above, the results obtained in the hemagglutination and hemolysis tests by various investigators vary greatly. Rothbard *et al.* (138), using the hemagglutination test, found a positive result in 92.2 per cent of tuberculous patients, whereas the control series did not give a single positive reaction. In the series of Schwartz *et al.* (141), tuberculous subjects gave in the same test positive reactions in 21.1 per cent only and healthy subjects in 7.1 per cent. These two series of results show extreme values, and the incidence of positive reactions in the hemagglutination test appears to range between 50 and 70 per cent in tuberculosis and between 10 and 30 per cent in the control series. Relatively few studies have been made of the hemolytic modification. However, the results appear to be close to those obtained with the hemagglutination test. The Meinicke test is generally regarded in the literature as one of the best serological tests for tuberculosis and for this reason I have selected it for the comparative test in this investigation on the value of the hemagglutination and hemolysis tests.

It was the aim of my investigation to study the following aspects of this subject:

1) Which of the above mentioned three serological tests for tuberculosis will give the best results?
2) Can a correlation be found between positive reactions and the clinical picture of the disease?
3) Can the value of the hemagglutination and hemolysis tests in the diagnosis of an active tuberculous process be increased by absorption techniques?
4) If two or all of the serological tests employed by me are positive, is this an indication of an active tuberculosis infection, and which combination of two of these three serological tests is the most serviceable in this respect?

AUTHOR'S INVESTIGATIONS

MATERIAL

The adult cases of tuberculosis were collected from the Municipal Tuberculosis Sanatorium, Helsinki, with the exception of a few cases which were from Aurora Hospital, Helsinki. In the childhood tuberculosis series, over one-half of the cases were from the Salpausselkä Sanatorium, Nastola, and the remaining cases from the Children's Clinic of the Helsinki Public Hospital and from the Muurola Sanatorium, Muurola. Other than tuberculous cases were collected partly from Aurora Hospital and partly from the Third Medical Clinic at the Kivelä Hospital, Helsinki. The sera of syphilitic patients were obtained from Wassermann samples taken in the Kumpula Hospital, Helsinki, and the samples of serum from pregnant women were received from the Maternity Clinic of the Midwifery School, Helsinki. The serum samples from healthy persons were collected among the personnel of the National Pension Institute and from healthy children under institutional care. The number of examined cases and their distribution according to disease is shown in table 4.

TABLE 4

SERIES OF CASES STUDIED BY THE MEINICKE, HEMAGGLUTINATION AND HEMOLYSIS TESTS FOR TUBERCULOSIS

	No. of Cases
Tuberculosis series:	
Adults with pulmonary tuberculosis	520
» » extrapulmonary tuberculosis	61
» » inactive tuberculosis	107
Children with tuberculosis	137
Total	825
Control series:	
Adults with non-tuberculous diseases	302
Patients with syphilis	33
Pregnant women	45
Healthy adults	271
Children, healthy or with non-tuberculous diseases	172
Total	823

TECHNIQUE OF THE SEROLOGICAL TESTS FOR TUBERCULOSIS

MEINICKE TEST

Since the purpose of this investigation was to study the diagnostic value of serological tests for tuberculosis, I utilised in the Meinicke test the saline solution series only, as the so-called soda series is designed chiefly for the continuous observation of the course of the disease. The antigens were obtained direct from Meinicke-Extrakte, Hamburg 21.

The Meinicke test was carried out as follows. Kahn test tubes 12—14 mm diameter, with a perfectly smooth bottom, were used. There were four antigen solutions, *i.e.*, water-soluble strong and weak antigens, alcohol-soluble tuberculosis antigen, and alcohol-soluble control antigen. The antigens were diluted with a 10 per cent saline solution, which before making of the test series was diluted with distilled water to 3.5 per cent. The sera were not inactivated after centrifugation. Into each of three test tubes there was pipetted 0.2 ccm of serum. At least 20 sera were tested at the same time, since this was technically easier and effected a saving of antigens. For preparation of the antigen dilutions, the antigens were pipetted into three tubes in the first row on the rack, placing 1 ccm of alcohol-soluble antigen into the first two tubes and 1 ccm of control antigen into the third tube. In the back row, 0.5 ccm of strong water-soluble antigen was placed in the first tube and 0.5 ccm of weak water-soluble antigen in the second tube. Then 9.5 ccm of 3.5 per cent saline solution was placed in the first two tubes in the back row and 10 ccm in the third tube. The contents of the first and second tubes in the back row were mixed thoroughly after addition of the saline solution. The tubes were placed in the water bath at +56° C for 5 min., after which the contents of the corresponding tubes in the front and back rows were mixed together well by pouring back and forth several times. The antigens were then placed once more in the same water bath for 2 min. While the antigen was still warm, 0.5 ccm of antigen was pipetted as quickly as possible from the first test tube into the tubes of sera in the first row, from the second tube into the tubes in the middle row, and from the third tube into the back row of tubes. After all the tubes had been shaken vigorously they were placed at room temperature for 24 hrs., read, shaken once more, and placed in the water bath at +37° C for 24 hours. For reading it is well to keep the tubes at an angle of about 45° for 10—15 min., as the degree of flow of the precipitate is a means of evaluating the intensity of the reaction. In a positive reaction the supernatant fluid is clear and the precipitate which has formed on the bottom is coarse-grained and does not run along the bottom when the tube is in an inclined position. In a moderately strong positive reaction the precipitate is less granular and extends over the entire bottom of the tube and usually does not run. A

weak positive reaction is indicated by some amount of precipitate around a blue knob which has formed on the bottom of the tube and which has run while the tube was inclined. In a doubtful reaction there are only a few grains around the blue knob, and in a negative reaction only this blue knob is seen in the centre of the tube bottom.

After carrying out Meinicke tests for some time I observed that the first reading was hardly usable for evaluation of the reaction, as it seemed positive in nearly all the tubes. On the other hand, the difference between a positive and a negative reaction was clearly visible in the second reading and the reaction could be judged more easily. On the whole, the reactions which in the first reading were strongly positive did not become weaker in the water bath, but the weak and doubtful reactions, which are very hard to estimate, frequently became more definitely positive or entirely negative. I interpreted as negative all the reactions in which either the first or the second reading showed a weak positive reaction in one of the tubes only, or a doubtful reaction in both tubes containing tuberculous antigen, since the reaction otherwise apparently would be too sensitive in comparison to the control cases.

In the few cases in which a positive reaction had developed also in the control tube I did not carry out the saturation test, as earlier experience had proved that the latter is rather unreliable.

PREPARATION AND TITRATION OF THE ANTIGEN FOR THE MIDDLEBROOK-DUBOS HEMAGGLUTINATION TEST AND THE MIDDLEBROOK HEMOLYSIS TEST

The antigen was prepared from tubercle bacillus strain H_{37} Rv grown on Sauton culture medium. The Sauton medium was prepared as follows: KH_2PO_4 0.5 gm, $MgSO_4$ 0.5 gm, Fe-ammon. citr. 0.05 gm, citric acid 2.0 gm, asparagine 4.0 gm, glycerin 60.0 gm, and distilled water 940 ccm.; pH 7.2. This medium, which is composed almost entirely of inorganic and organic salts, was chosen in order to eliminate the non-specific action of proteins possibly present in the culture medium. The strain was grown on the culture medium for 6 weeks. The surface was then covered by a thick, wrinkled membrane and the underlying fluid was brownish. To prepare the antigen the bacillary mass was removed by filtration through ordinary filter paper, after which the fluid was passed once more through a Seitz bacterial filter. The liquid was concentrated by

lyophilisation to about 20-fold, *i.e.*, 5 litres of culture medium were reduced to about 250 ccm of dark brown liquid of the consistency of syrup. To remove the glycerin and the salts the liquid was dialysed for 3 days, first against tap water and then against distilled water. The small amount of sediment that may have formed during dialysation was removed by filtration. The dialysate was then lyophilised, leaving a yellowish powder. The yield from 5 litres of culture medium was about 5—6 gm of powder for use as antigen.

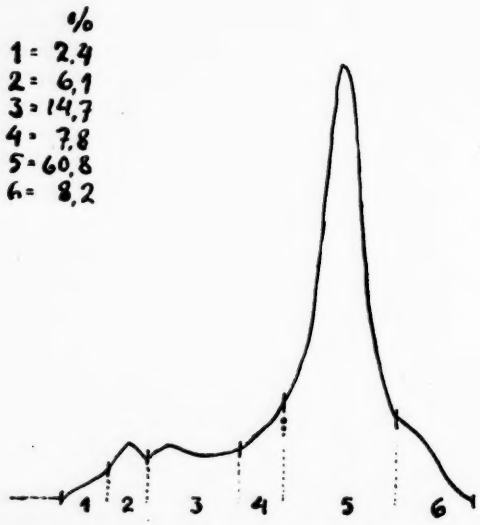

Fig. 1. — Micro-electrophoretic pattern of the antigen, using the Antweiler apparatus. Veronal buffer, pH 8.6, ionic strength 0.12, length of run 15 min., current 55 V., 1.7 mA. Antigen dialysed against the buffer for 2 hrs. before electrophoresis.

About 40 per cent of the dry weight of the powder was protein, determined by the biuret test, and about 50—60 per cent were hexoses which gave the orcinol reaction. According to microelectrophoresis, the antigen appears to have 5 or 6 components. The curve in Fig. 1 shows the relative magnitude of these components. The largest component, the fifth, migrated slowly.

The strength of the antigen was determined by sensitisation of sheep red cells with varying amounts of antigen and by parallel titrations by the hemagglutination and the hemolysis methods of three sera from tuberculous patients and the serum of one healthy

subject. For sensitisation I used 5, 10, 15, 20, 25 and 30 mg of antigen for each 0.1 ccm of sheep red cells.

Table 5 shows the results obtained in the hemagglutination and hemolysis tests when variable amounts of antigen were used for

TABLE 5

RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS USING SHEEP RED CELLS SENSITISED WITH VARIABLE AMOUNTS OF ANTIGEN

Amount of Antigen per 0.1 ccm of Sheep Red Cells	Hemagglutination Titres				Hemolysis Titres			
	Serum No.				Serum No.			
	1	2	3	Neg. Contr.	1	2	3	Neg. Contr.
5 mg	1: 2	1: 32	1: 32	0	1: 4	1: 2	1: 32	0
10 mg	1: 8	1: 64	1: 64	0	1: 8	1: 32	1: 32	0
15 mg	1: 8	1: 64	1: 32	0	1: 8	1: 32	1: 32	0
20 mg	1: 8	1: 64	1: 32	0	1: 8	1: 32	1: 32	0
25 mg	1: 8	1: 64	1: 32	0	1: 8	1: 32	1: 32	0
30 mg	1: 8	1: 64	1: 32	0	1: 8	1: 16	1: 32	0

sensitisation of the sheep red cells. On the basis of these results, 10 mg of antigen per 0.1 ccm of sheep cells was selected as the optimum amount. Obviously, larger amounts may also be used, but in the hemolysis test in particular the use of very large amounts of antigen are not advisable since they cause hemolysis of the sheep cells during the sensitisation procedure.

Since several kinds of tuberculins and purified tuberculin proteins and polysaccharides have been used in the past as antigens in the hemagglutination and hemolysis tests it was considered necessary to compare some of these antigens and the one prepared by me. In table 6 will be seen the titres obtained after sensitisation of sheep red cells with 4 times concentrated Lederle tuberculin, »Berna» tuberculin from a human strain, and Danish purified protein derivative PPD Copenhagen.

Lederle tuberculin in dilution 1: 15 gave definitely weaker hemagglutination and hemolysis reactions. My own antigen gave an approximately equally strong reaction in the hemagglutination test as Sauton tuberculin »Berna» but a definitely stronger reaction in the hemolysis test. Gernez-Rieux and Tacquet (108) have demonstrated that different batches of I.P. 48 give slightly diverging titres

TABLE 6

RESULTS OF COMPARATIVE TITRATIONS WITH OWN ANTIGEN AND SOME COMMONLY USED TUBERCULINS

Antigen	Hemagglutination Titres					Hemolysis Titres				
	Serum No.					Serum No.				
	1	2	3	Pos. Contr.	Neg. Contr.	1	2	3	Pos. Contr.	Neg. Contr.
Lederle 1: 12	1: 2	1: 16	1: 32	1: 8	0	1: 2	1: 32	1: 32	1: 16	0
Lederle 1: 15	1: 2	1: 16	1: 16	1: 4	0	0	1: 16	1: 16	1: 16	0
Sauton Tuberculin »Berna»	1: 16	1: 64	1: 64	1: 8	0	0	1: 16	1: 32	1: 32	0
PPD Copenhagen 1 mg/0.1 ccm sheep red cells ..	1: 2	1: 16	1: 32	1: 8	0	0	1: 16	1: 16	1: 16	0
My antigen 10 mg/0.1 ccm sheep red cells ..	1: 16	1: 64	1: 64	1: 8	0	1: 8	1: 32	1: 64	1: 32	0

for the same sera. They also reached the conclusion that I.P. 48 is more suitable for the hemolysis test, whereas the polysaccharide fraction of I.P. 48 is better suited for the hemagglutination test.

HEMAGGLUTINATION TEST

It is not necessary to carry out the hemagglutination test immediately, as the sera will stand storage for some time without alteration in the hemagglutination titre. However, I performed the tests in most cases within one week from drawing of the blood samples. Gernez-Rieux and Tacquet (108) observed that the sera could be kept for 8 days at +4° C, and Rothbard (139) was able to store serum in the refrigerator for one year without change in the titre. Prior to the test I inactivated the serum by keeping it at +56° C for ½ hr. Hilson and Elek (116) did not at first inactivate the polysaccharide antigen used by them for red cell sensitisation but later they considered inactivation preferable. Bunn *et al.* (94) carried out the inactivation at +60° C.

For this test I absorbed the serum once with washed sheep cells, using 0.2 ccm of cells per 2 ccm of serum dilution 1: 2 and storing in the refrigerator overnight. On the following morning the cells were centrifuged down and the supernatant absorbed serum was removed with a pipet. Madsen (126) considered one absorption sufficient, but Sievers *et al.* (144) absorbed

the serum six times, since in their opinion this helped to reduce the number of non-specific reactions. I followed the latter procedure in a part of these cases, as will be seen later. In some rare cases in which absorption had not been sufficient, *i.e.*, in which the control tube showed agglutination, the absorption was repeated. If O Rh— cells are used, absorption of course is unnecessary.

The sheep red cells used in the test tube series and in sensitisation were always from the same sheep and fresh. Adcock *et al.* (87) obtained a more permanent result with human O Rh— cells but the titre was lower when sheep cells were used. The same conclusion was reached by Bunn *et al.* (94). The suitability of various red cells for sensitisation was investigated by Gernez-Rieux and Tacquet (108), who found human, sheep, chicken and horse cells best suited for this purpose. Another reason for the use of sheep cells only in my investigation was the poor suitability of human red cells for the hemolysis test, which I always carried out parallel to the hemagglutination test.

Before sensitisation the sheep red cells were washed three times. They were then suspended in buffered saline solution made up as follows: $Na_2HPO_4 \cdot 12\ H_2O$ 0.093 gm, KH_2PO_4 0.17 gm, NaCl 10.0 gm, formaldehyde 0.5 ccm, distilled water 1000 ccm; pH 6.0—6.1. The cells were used in the amount of 0.5 ccm per 10 ccm of buffer solution and the amount of antigen was 10 mg per 0.1 ccm of packed sheep red cells. During sensitisation the red cell suspension was kept on the water-bath at +37° C for 2 hrs. and the tubes were shaken at intervals of 15 min. Yaoi *et al.* (157) recommended sensitisation either at +37° C for 2 hrs. or at +20° C for 3 hrs. Madsen (126) observed that red cells were only partly sensitised in 24 hrs. at +4— +6°C, but completely sensitised in 4 hrs. at room temperature and in 1 hr. at +37° C. Gernez-Rieux and Tacquet (108) found that 1 hr. is usually sufficient for sensitisation but they used 2 hrs. Boyden (92) regarded that red cell sensitisation is clearly dependent on the acidity of the solution and considered pH 6.4 suitable, but he also used at the same time a phosphate-buffered saline solution. Gernez-Rieux and Tacquet studied the effect of acidities between pH 4.2—8.6 and found no effect within these limits.

As was mentioned above, I used 10 mg of antigen per 0.1 ccm of packed red cells. Fischer (100) also considered a cell/antigen ratio of 1: 10—1: 20 suitable. Sohier *et al.* (146) used 1: 20, Middlebrook (134) 1: 40 for Lederle tuberculin, and Lewin *et al.* (124) also 1: 40 whereas the ratio 1: 80 was used by Maillard and Gagliardo (128). In the hemagglutination test I used a 0.5 per cent suspension of sensitised sheep red cells. Boyden (92) used a 2.5 per cent suspension but placed only 0.05 ccm in each tube. Gernez-Rieux and Tacquet used a 0.5 per cent suspension of sensitised cells and added into the test tube the same amount of sensitised cells as there was serum dilution.

After sensitisation the cells were washed three times with physiological saline solution and a 0.5 per cent red cell suspension was then prepared. Into the first tube in the series 0.2 ccm of serum dilution 1: 2 was pipetted, and

into each of the next five tubes 0.2 ccm of saline solution. Into the second tube was added 0.2 ccm of the serum dilution, 0.2 ccm was transferred from the second to the third tube, and so on down the line, thus obtaining dilutions of 1: 2, 1: 4 . . . . 1: 64. For each serum a control tube was made with unsensitised 0.5 per cent sheep cell suspension to check the absorption, as well as a saline solution and a positive and a negative serum control. The positive serum controls were three tuberculotic sera of known titres, which had been stored frozen, and the negative controls were sera which had been examined earlier and serum from a member of the laboratory staff.

HEMOLYSIS TEST

It was demonstrated by Lucentini and Boiswert (125) that human red cells give considerably lower titres than sheep cells in the hemolysis test. Gernez-Rieux and Tacquet (108) found sheep cells best in comparative tests with human, ox, sheep, chicken, horse, guinea pig and rabbit red cells in which they employed BCG immune serum and as antigen polysaccharide fraction of I.P. 48. Middlebrook (133), Gernez-Rieux and Tacquet (108), Lucentini and Boisvert (125) and Sherris (143) used 0.5 per cent sensitised sheep red cells in the hemolysis test, Fischer used 5 per cent, Maillard and Gagliardo (127) 2.5 per cent and Mollov and Kott (135) 0.3 per cent. I used in this investigation a 2.5 per cent suspension of sheep red cells sensitised with the same amount of antigen and under the same sensitisation conditions and time as in the hemagglutination test.

The complement was pooled serum drawn by heart puncture from several guinea pigs. The cells were removed by centrifugation and the serum was stored at +4° C. The complement was 3—4 days old at the most and a titration was carried out each time before its use. The titration of this complement was made according to Wadsworth (166), using unsensitised sheep cells, a known amboceptor in varying dilutions (usually 1: 750 or 1: 1000) and the complement diluted according to the formula. The highest dilution of complement that is capable of producing a 50 per cent hemolysis in the sheep red cell suspension was regarded as the unit of complement. In most cases this required 0.1 ccm of complement in dilution 1: 40. Middlebrook, and Gernez-Rieux and Tacquet employed in their hemolysis tests 3 times the unit of complement. Like Maillard and Gagliardo (128) I used 4 times the unit, *i.e.*, 0.1 ccm of comple-

ment, usually in dilution 1: 10. The dry complement recommended by Popp (136) has not been available to me.

Gernez-Rieux and Tacquet determined the amount of tuberculin required to produce hemolysis in a known immune serum of titre 1: 16. The following amounts of different tuberculins are needed to sensitise 0.1 ccm of sheep red cells:

Tuberculin I.P. 48	40	units
» » », polysaccharide-rich	200	»
PPD Copenhagen	4,000	»
Tuberculin G.T. Hoechst	4,000	»
» »Berna», from human strain	200	»
» » » bovine »	2,000	»
PPD Weybridge	0.02	mg
Tuberculin Lederle, 4 times concentrated	0.5	ccm

Thus definitely smaller amounts of tuberculin are required to sensitise the red cells for the hemolysis reaction than for the hemagglutination reaction. An excess amount of tuberculin is generally used for sensitisation. Gernez-Rieux and Tacquet (108) used 100—250 times the amount required according to titration. However, since the resistance of the red cells is reduced somewhat by sensitisation, a number of titrations must be made to preclude an untoward effect on the results of the reaction from this reduction of sensitisation.

It is also to be taken into consideration that the amount of complement used should not to be so great as to have a hemolysing effect *per se* on the sensitised red cells. In addition to a determination of the strength of the complement, titration was also carried out to determine the dilution in which the complement has a hemolytic action on ordinary and sensitised sheep red cells. A further titration was made using a given volume of amboceptor but with variable amounts of complement to determine whether hemolysis of both sensitised and unsensitised cells occurs in approximately the same complement dilution, *i.e.*, to determine that the resistance of the red cells was not reduced by sensitisation. The results of these titrations are shown in figures 2—5.

It will be noted from titrations 1 and 2 that when complement was diluted 1: 10 hardly any difference was seen in the hemolysis of sensitised and of unsensitised sheep red cells. Titrations 3 and

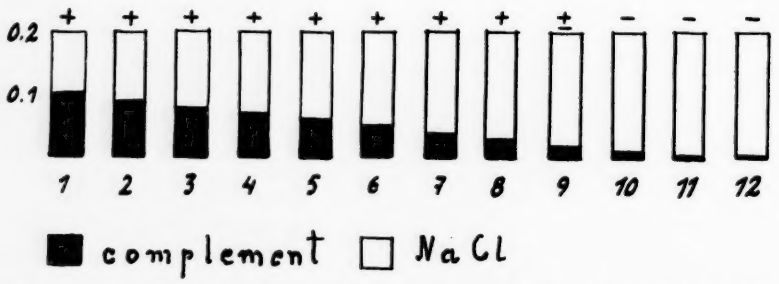

Fig. 2. — Results of titration I, using 0.2 ccm of a 2.5 per cent suspension of sensitised sheep red cells, 0.1 ccm of amboceptor 1: 1000, saline solution, and non-absorbed complement 1: 10.

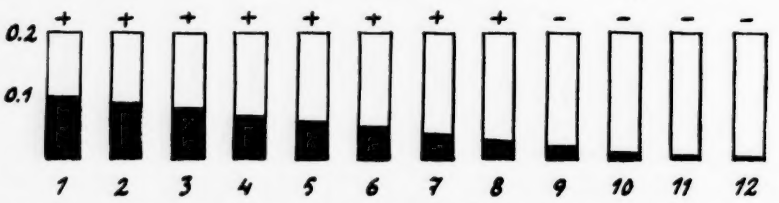

Fig. 3. — Results of titration II, using 0.2 ccm of a 2.5 per cent suspension of unsensitised sheep red cells, 0.1 ccm of amboceptor 1: 1000, saline solution, and non-absorbed complement 1: 10.

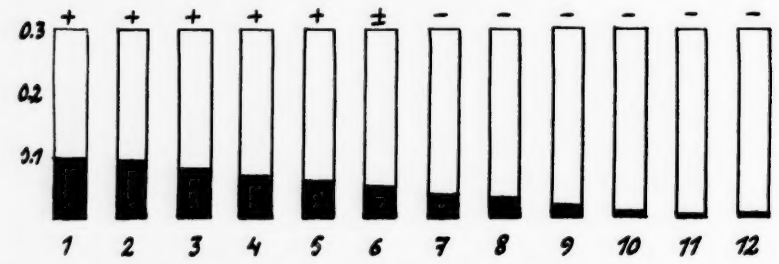

Fig. 4. — Results of titration III, using 0.2 ccm of a 2.5 per cent suspension of sensitised sheep red cells, saline solution, and undiluted and non-absorbed complement.

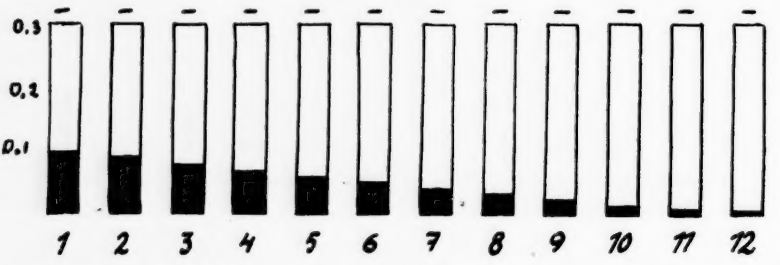

Fig. 5. — Results of titration IV, using 0.2 ccm of a 2.5 per cent suspension of unsensitised sheep red cells, saline solution, and undiluted non-absorbed complement.

4 show that undiluted complement had a hemolytic action on sensitised cells but not on normal sheep cells. This hemolytic capacity clearly disappeared, however, before the dilution was reached which was used in the hemolysis test itself, for the amount of complement used in most cases was the same as the amount of undiluted complement in the tenth test tube. A further titration using sensitised sheep red cells was made with a serum of a known titre and 2, 4, 6 and 8 units of complement. Usually I did not in the hemolysis test utilise complement absorbed with sheep cells, since in the titrations the complement did not have a hemolytic capacity against sheep cells, and as, furthermore, no difference was seen in the titres in parallel tests with a number of sera with absorbed and nonabsorbed complement.

In the test series proper I first carried out in each case a preliminary test to determine whether the serum contained antibodies which would cause hemolysis of sensitised red cells. Since the amount of serum frequently was inadequate for all three serological reactions I only used in the preliminary test 0.1 ccm of a serum dilution 1: 2, adding to it 0.2 ccm of a 2.5 per cent sensitised sheep red cell suspension, 0.1 ccm of complement and 0.1 ccm of physiological saline solution. The controls were the same serum, unsensitised sheep red cells and complement, sensitised sheep cells and complement without serum, and known positive and negative control sera. The tubes were shaken well, placed in the refrigerator for 1 hr., then in the water bath for ½ hr. at +37° C, and while in the latter shaken at 5 min. intervals. The preliminary test was interpreted as positive if the serum showed 50 per cent or greater hemolysis. Sera in which slight or no hemolysis occurred were considered negative and were not used in the final titration. Of the remaining sera the usual dilution series of 1: 2, 1: 4 . . . 1: 256 were made, and the serum dilution in which 50 per cent or higher hemolysis was observable was regarded as the borderline.

RESULTS OF SEROLOGICAL TESTS

EXPERIMENTS WITH GUINEA PIGS

Hemagglutination and hemolysis tests were carried out with 35 healthy guinea pigs and 35 guinea pigs infected with tubercle bacilli. Inoculation was made by a subcutaneous injection of 0.2 mg living bacilli of strain H_{37} Rv. Blood was withdrawn 4—6 weeks after infection and the infected animals were autopsied. Miliary tuberculosis was found in all these animals. Table 7 shows the results of this experiment.

TABLE 7

RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS WITH SERA OF HEALTHY AND TUBERCULOUS GUINEA PIGS

Test	Titres									
	< 1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥1:256	
Hemagglutination										
Healthy guinea pigs	35	—	—	—	—	—	—			
	35=100 %									
Tuberculous guinea pigs	3	—	5	9	6	9	3			
	17=48.6 %				18=51.4 %					
Hemolysis										
Healthy guinea pigs	31	2	2	—	—	—	—	—	—	
	35=100 %									
Tuberculous guinea pigs	1	—	5	4	10	7	4	2	2	
	10=28.6 %				25=71.4 %					

All the 35 healthy guinea pigs had a hemagglutination titre below 1: 2. In the hemolysis test 31 animals had a titre below 1: 2, 2 animals 1: 2 and 2 animals 1: 4. The result corresponds fully with the results obtained by Gernez-Rieux and Tacquet (104), referred to above. If titre values of 1: 16 and higher are regarded as positive in the hemagglutination and hemolysis reactions, 18 of the 35 tuberculous guinea pigs (51.4 per cent) gave positive hemagglutination reactions and 25 (71.4 per cent) positive hemolysis reactions. Gernez-Rieux and Tacquet (108) examined the sera of 193 guinea pigs which were injected with various samples sent for examination. Autopsy showed tuberculous changes in 67 animals, 36 of which (53.7 per cent) had a hemagglutination titre of 1:16 or more.

HEALTHY ADULT SERIES

All test subjects in the healthy adult series which was used as control were examined by x-ray of the lungs and the tuberculin test. Personnel health cards were also available and in most cases covered a period of several years. Thus four persons were excluded from the series because of pleuritis 1—2 years previously, one person because of recent treatment for pulmonary tuberculosis, and

one because of acute pulmonary tuberculosis found in the x-ray examination. Further there were excluded a case of acute rheumatoid arthritis and two cases of pregnancy. On the other hand, I included 7 persons in whom traces of previous tuberculosis infection were seen in the form of either calcification in the lungs or adhesion at the costophrenic angle.

TABLE 8

RESULTS OF SEROLOGICAL TESTS FOR TUBERCULOSIS WITH SERA OF HEALTHY ADULTS

Test	Titres									Total
	< 1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥ 1:256	
Hemagglutination	60	44	54	40	20	4	2			=224 } =271
	19*	8*	6*	10*	2*	—*	2*			=47* }
	241 = 88.9 %				30 = 11.1 %					
Hemolysis	156	18	5	13	11	9	10	2	—	=224 } =271
	34*	1*	1*	1*	4*	2*	—	1*	3*	=47* }
	229 = 84.5 %				42 = 15.5 %					
Meinicke	negativ 254 = 94.5 %				positive 15 = 5.5 %				? 2	=271

In table 8 will be seen the distribution of the results for the total healthy adult material in the three serological tests used. In the hemagglutination and hemolysis tests the titres are also stated. *As also in the later tables in this investigation, the reaction was interpreted as positive if the titre was 1: 16 or higher.* Tuberculin-negative cases are indicated by *.

Expressed in percentages, the smallest proportion of positive reactions in this control series was seen in the Meinicke test, in which they occurred in 5.5 per cent, then in the hemagglutination test, at 11.1 per cent, and the most in the hemolysis test, at 15.5 per cent. Koulumies and Haahti (122) obtained an incidence of 35.3 per cent positive reactions in the hemagglutination test but they regarded a titre of 1: 8 already positive. Gernez-Rieux and Tacquet (108), whose series was the largest I have found in the literature for the hemagglutination and hemolysis tests, obtained, in using I.P. 48 as antigen, positive reactions in 9.2 per cent of 259 healthy subjects in hemagglutination test and in 12.1 per cent of 172 healthy

TABLE 9

RESULTS OF SEROLOGICAL TESTS FOR TUBERCULOSIS USING SERA OF SUBJECTS WITH NON-TUBERCULOUS DISEASES AND CONDITIONS

Disease or Condition	No. of Cases		Titres									Meinicke		
			< 1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥ 1:256	+	—	?
Diseases of central and periph. nervous system	17	Aggl.	1	2	2	7	2	3	—			7	9	1
		Hemol.	7	2	1	—	7	—	—	—	—			
Cardiac and vascular diseases	34	Aggl.	4	3	14	7	5	1	—			6	27	1
		Hemol.	17	1	4	4	2	4	1	1	—			
Non-tuberculous pulmonary diseases	50	Aggl.	15	5	11	11	5	3	—			19	28	3
		Hemol.	23	2	3	12	7	1	1	1	—			
Diseases of alimentary tract	15	Aggl.	6	1	3	4	1	—	—			2	13	—
		Hemol.	10	—	—	2	1	1	1	—	—			
Liver and bile-duct diseases	7	Aggl.	1	2	1	1	2	—	—			2	5	—
		Hemol.	2	—	2	—	—	1	2	—	—			
Non-tuberculous pleural diseases	8	Aggl.	3	—	1	1	3	—	—			2	6	—
		Hemol.	2	—	—	1	4	—	1	—	—			
Renal diseases	4	Aggl.	1	1	2	—	—	—	—			—	4	—
		Hemol.	3	—	—	—	—	1	—	—	—			
Anemias	3	Aggl.	1	—	—	1	1	—	—			2	1	—
		Hemol.	2	—	—	—	—	1	—	—	—			
Metabolic diseases	7	Aggl.	1	—	3	—	1	2	—			3	4	—
		Hemol.	2	—	—	2	1	2	—	—	—			
Malignant tumours	10	Aggl.	2	3	1	4	—	—	—			2	8	—
		Hemol.	8	1	—	—	—	1	—	—	—			
Syphilis	33	Aggl.	8	7	7	5	6	—	—			3	4	26
		Hemol.	26	1	—	1	2	—	2	1	—			

Rheumatic fever	6	Aggl.	2	1	2	1	—	—	—			3	3	—
		Hemol.	4	—	—	—	1	1	—	—	—			
Chronic rheumatoid arthritis	50	Aggl.	9	8	16	9	7	—	1			4	45	1
		Hemol.	30	11	2	2	2	2	1	—	—			
Other diseases of bones and joints	3	Aggl.	2	—	—	—	—	—	1			1	2	—
		Hemol.	2	—	—	—	—	—	1	—	—			
Gynecological diseases	6	Aggl.	4	1	1	—	—	—	—			2	4	—
		Hemol.	4	1	1	—	—	—	—	—	—			
Typhoid and paratyphoid fever	6	Aggl.	3	2	—	—	1	—	—			1	5	—
		Hemol.	5	—	1	—	—	—	—	—	—			
Scarlatina	58	Aggl.	5	9	13	11	14	5	1			23	34	1
		Hemol.	20	7	9	7	6	6	1	2	—			
Tonsillitis	12	Aggl.	—	2	3	4	2	1	—			4	7	1
		Hemol.	2	2	—	6	2	—	—	—	—			
Diphtheria	1	Aggl.	—	—	—	—	—	1	—			1	—	—
		Hemol.	—	—	—	—	—	—	1	—	—			
Jaundice (hepatitis ac.)	3	Aggl.	1	1	1	—	—	—	—			—	3	—
		Hemol.	2	—	—	1	—	—	—	—	—			
Mononucleosis	2	Aggl.	—	—	—	2	—	—	—			—	2	—
		Hemol.	1	—	1	—	—	—	—	—	—			
Pregnancy	45	Aggl.	9	6	2	13	9	6	—			20	22	3
		Hemol.	14	2	1	4	4	6	7	5	2			
Total	380	Aggl.	78	54	83	81	59	22				108	235	37
			299 = 78.7 %				81 = 21.3 %					31.5 %	68.5 %	
		Hemol.	186	30	25	42	39	27	19	10	2			
			283 = 74.5 %				97 = 25.5 %							

subjects in hemolysis test. In my tuberculin-negative cases the Meinicke test was positive in 4.3 per cent, the hemagglutination test in 8.5 per cent, and the hemolysis test in 21 per cent. The hemolysis test thus gave a positive reaction more frequently in the tuberculin-negative persons than in tuberculin-positive persons.

NON-TUBERCULOUS DISEASES AND CONDITIONS IN ADULTS

In selecting my control series I made a special effort to obtain a large number of cases of the types that usually give confusing results in serological tests, such as acute infectious diseases, syphilis, rheumatoid arthritis and pregnancy.

Although in the listing of the total material in table 4 the cases of pregnancy and syphilis were grouped separately, I have combined them in table 9 because of the small number of cases concerned.

It is noted from this table that 108 cases (31.5 per cent) of 343 cases of non-tuberculous diseases or pregnancy gave a positive Meinicke reaction. In the hemagglutination test, positive reactions were seen in 81 out of 380 cases (21.3 per cent) and in the hemolysis test in 97 cases (25.5 per cent).

In the Meinicke test there was a high incidence of positive reactions in three groups in particular, *i.e.*, in non-tuberculous diseases of the lungs, in which 19 out of 47 cases (40.4 per cent) had positive reactions, in scarlatina, with 23 positive reactions in 57 cases (40.4 per cent), and in pregnancy, in which the reaction was positive in 20 out of 42 cases (47.6 per cent). A comparison with the results reported in the literature shows that the 113 positive reactions in Böhne's (12) control series included 61 cases of scarlatina. Leitner (47) observed that pregnancy frequently gave a positive Meinicke reaction. On the other hand, the large number of positive reactions in the non-tuberculous diseases of the lungs in my control series was exceptional. In syphilis, as is usual, the control tube also showed a positive reaction in nearly all cases.

The hemagglutination and hemolysis tests made in the series of non-tuberculous diseases and conditions showed the largest number of positive reactions in scarlatina and pregnancy. The 58 cases of scarlatina gave 20 positive reactions (34.5 per cent) in the hemagglutination test and 15 (25.9 per cent) in the hemolysis test. In 42 cases of pregnancy the hemagglutination test was positive in 15

cases (33.3 per cent) and the hemolysis test in 24 (53.3 per cent). All subjects with present or previous tuberculosis were excluded from the pregnancy group. Gernez-Rieux and Tacquet (108) are of the opinion that gestation does not cause positive hemagglutination reactions, whereas Howard *et al.* (118) obtained a positive reaction in 57 per cent of a series of 37 pregnant women. Four months after delivery only 2 positive hemagglutination reactions were seen in the 12 cases which could be followed up.

ADULT CASES OF PULMONARY TUBERCULOSIS

Distribution of Results According to Serological Test

The series in this study comprised 520 cases of clinically active pulmonary tuberculosis. In 514 cases these were patients in the Helsinki Municipal Tuberculosis Sanatorium and 6 were cases of active pulmonary tuberculosis diagnosed at the Aurora Hospital. The case history and roentgenograms were available to me in every case. The Meinicke, hemagglutination and hemolysis tests were carried out in all cases and the results for the entire series in each test will be seen in table 10.

TABLE 10

RESULTS OF SEROLOGICAL TESTS FOR TUBERCULOSIS USING SERA OF SUBJECTS WITH PULMONARY TUBERCULOSIS

No. of Cases	Test	Positive		Negative		Meinicke?
		No.	%	No.	%	
520	Meinicke	302	58.9	211	41.1	7
520	Hemagglutination	259	49.8	261	50.2	
520	Hemolysis.....	284	54.6	236	45.4	

Even when the 7 cases in which also the control tube was positive in the Meinicke test are excluded, the highest incidence of positive reactions in active pulmonary tuberculosis, *i.e.*, 58.9 per cent, was obtained by this method. The hemolytic test for tuberculosis gave, at 54.6 per cent, the next to the highest incidence, and the lowest was seen in the hemagglutination test, in which it was 49.8 per cent. A comparison of the incidence of positive reactions obtained in active pulmonary tuberculosis by the Meinicke test in

my series with the incidence of 76.8 per cent obtained in Finland by Koulumies (41) reveals a considerably lower incidence in my series. My low figure is undoubtedly influenced also by the inclusion of all doubtful reactions in the negative group. On the other hand, my results in the hemagglutination test conform fairly well with those obtained in Sweden by Sievers *et al.* (144), who found positive reactions in tuberculosis in 53.4 per cent of cases. Koulumies and Haahti (122), on the other hand, had positive hemagglutination reactions in 71.8 per cent; however, a titre of 1: 8 was interpreted by them as a positive reaction. I have found in the literature no reports on results of the hemolysis test in Scandinavia.

TABLE 11

RESULTS OF HEMAGGLUTINATION TEST IN DIFFERENT TYPES OF PULMONARY TUBERCULOSIS

Type of Process	No. of Cases	Titres	
		<1:2 1:2 1:4 1:8	1:16 1:32≥1:64
Bilateral exudative.....	202	16 10 27 31 84=41.6%	46 28 44 118=58.4%
Unilateral exudative	84	7 3 11 13 34=40.5%	19 14 17 50=59.5%
Apical or upper field exudative	70	17 10 8 13 48=68.6%	12 4 5 22=31.4%
Extensive productive.....	7	1 — 1 1 3=42.9%	2 2 — 4=57.1%
Apical or upper field productive	54	14 2 6 11 33=61.1%	12 6 3 21=38.9%
Extensive exudative-productive	45	2 4 8 9 23=51.1%	10 8 4 22=48.9%
Apical or upper field exudative -productive	25	4 5 5 4 18=72.0%	1 1 5 7=28.0%
Tuberculoma	6	1 1 1 1 4=66.7%	1 1 — 2=33.3%
Pulmonary and extrapulmonary	27	4 1 3 6 14=51.9%	5 4 4 13=48.1%
Total	520	66 36 70 89 261=50.2%	109 68 82 259=49.8%

Correlation of Serological Reactions and the Type and Extent of the Pulmonary Process

Since the incidence of positive reactions in different types and varying extent of pulmonary tuberculosis is a subject of interest, I have also distributed the test results on this basis according to the principles published by Forsman and Larmola (163). Table 11 shows this distribution, subdivided also according to titre.

TABLE 12

RESULTS OF HEMOLYSIS TEST IN DIFFERENT TYPES OF PULMONARY TUBERCULOSIS

Type of Process	No. of Cases	Titres								
		<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≧1:256
Bilateral exudative	202	38	9	14	18	25	24	34	15	25
		79=39.1 %				123=60.9 %				
Unilateral exudative	84	21	2	3	9	8	8	15	9	9
		35=41.7 %				49=58.3 %				
Apical or upper field exudative	70	23	5	4	7	4	12	9	2	4
		39=55.7 %				31=44.3 %				
Extensive productive	7	2	1	—	—	1	1	1	1	—
		3=42.9 %				4=57.1 %				
Apical or upper field productive	54	14	3	7	6	7	6	8	2	1
		30=55.6 %				24=44.4 %				
Extensive exudative-productive	45	6	3	6	1	8	11	3	3	4
		16=35.6 %				29=64.4 %				
Apical or upper field exudative-productive	25	12	1	1	3	1	2	2	1	2
		17=68.0 %				8=32.0 %				
Tuberculoma	6	3	1	—	—	—	1	—	1	—
		4=66.7 %				2=33.3 %				
Pulmonary and extrapulmonary	27	7	2	2	2	4	4	3	1	2
		13=48.1 %				14=51.9 %				
Total	520	126	27	37	46	58	69	75	35	47
		236=45.4 %				284=54.6 %				

There appears to be a definite correlation between a positive hemagglutination reaction and the extent of the pulmonary process, since the largest number of positive reactions occurred in cases of extensive bilateral and unilateral exudative pulmonary tuberculosis, whereas in all forms of tuberculosis in the apex or the upper field the incidence of positive reactions was considerably lower. The same observation made by Hollander *et al.* (117).

The hemolysis test, likewise, gave the greatest number of positive reactions in the cases with an extensive pulmonary process. The highest incidence, 64.4 per cent, was seen in extensive pulmonary tuberculosis of the mixed type. In comparison with the results of Gernez-Rieux and Tacquet (108) the percentage of positive reactions in my series is definitely lower. Using I.P. 48 as antigen these workers obtained positive reactions in the hemolysis test in 88.8 per cent of bilateral and 80.9 per cent of unilateral exudative processes; when the polysaccharide fraction of I.P. 48 was employed as antigen the incidences were 62.9 and 58.2 per cent, respectively. The latter two figures are fairly close to my results.

The largest number of positive Meinicke reactions occurred in patients with extensive bilateral exudative pulmonary tuberculosis. Contrary to the results obtained in the hemagglutination and hemolysis tests, there were comparatively many positive reactions in the mixed type of apical or upper field processes, in which they occurred in 60.0 per cent.

TABLE 13

RESULTS OF MEINICKE TEST IN DIFFERENT TYPES OF PULMONARY TUBERCULOSIS

Type of Process	Positive		Negative		Doubtful	Total
	No.	%	No.	%	No.	
Bilateral exudative	147	72.2	51	27.8	4	202
Unilateral exudative	43	51.8	40	48.2	1	84
Apical or upper field exudative	25	35.7	45	64.3	—	70
Extensive productive	4	57.1	3	42.9	—	7
Apical or upper field productive	27	50.0	27	50.0	—	54
Extensive exudative-productive	23	52.3	21	47.7	1	45
Apical or upper field exudative-productive	15	60.0	10	40.0	—	25
Tuberculoma	3	50.0	3	50.0	—	6
Pulmonary and extrapulmonary	15	57.7	11	42.3	1	27
Total	302	58.9	211	41.1	7	520

Correlation of Serological Reactions and the Duration of the Disease and the Sedimentation Rate

I have made a further distribution of the test results to study whether cases with a long history give positive reactions more frequently than cases of short duration. This distribution is shown in table 14.

TABLE 14

RESULTS OF SEROLOGICAL TESTS IN CASES OF PULMONARY TUBERCULOSIS, DISTRIBUTED ACCORDING TO DURATION OF DISEASE IN YEARS

Test	0—1			1—3			3—5			5			Total
	+	—	?	+	—	?	+	—	?	+	—	?	
Meinicke	85	90	2	82	66	2	38	31	2	95	26	1	=520
Hemagglutination	68	109		81	69		39	32		72	50		=520
Hemolysis	80	97		84	66		43	28		76	46		=520

It appears that in all the serological tests used the incidence of positive reactions clearly increased with the duration of the disease. This was particularly evident in the Meinicke test, in which 78.5 per cent of the cases of pulmonary tuberculosis with a history of over 5 years gave positive reactions.

The correlation between the results of the serological tests and the sedimentation rate is seen in table 15. I have excluded from this

TABLE 15

RESULTS OF SEROLOGICAL TESTS IN CASES OF PULMONARY TUBERCULOSIS, DISTRIBUTED ACCORDING TO SEDIMENTATION RATE

Test	≤9 mm			10—29 mm			30—59 mm			≧60 mm			Total
	+	—	?	+	—	?	+	—	?	+	—	?	
Meinicke	38	63	1	89	68	1	90	50	1	80	30	4	=515
Hemagglutination	35	67		77	81		69	72		75	39		=515
Hemolysis	45	57		84	74		89	52		63	51		=515

table 5 cases, consisting of 2 patients with syphilis, 1 with rheumatoid arthritis, 1 with cancer of the lung, and 1 with lymphatic leucemia, since the sedimentation rate appeared to be more greatly dependent on these diseases than on the pulmonary tuberculosis.

On the basis of this table it seems probable that the positive reactions in serological tests definitely increase as the sedimentation rate becomes elevated. In studying the relationship between the hemagglutination test and the sedimentation rate Koulumies and Haahti (122) observed in 125 cases and Gernez-Rieaux and Tacquet (108) in 1,405 cases that the number of positive reactions increased in proportion to the elevation in the sedimentation rate.

Serological Tests in Pulmonary Tuberculosis with Cavity Formation

I did not distribute the cases of pulmonary tuberculosis according to the presence or absence of tubercle bacilli in the sputum, since they frequently disappear from the sputum during chemotherapy and reappear in some cases after discontinuance of the chemotherapeutic measures. I considered it preferable to segregate those cases in which the roentgenogram revealed cavity formation. The results of the serological tests in these cases are shown in table 16.

TABLE 16

RESULTS OF SEROLOGICAL TESTS IN CASES OF PULMONARY TUBERCULOSIS WITH CAVITY FORMATION

Test	Titres										Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	≥1:256	
Hemagglutination	19	11	32	33	55	37	50				=237
	95=40.1%				142=59.9%						
Hemolysis	40	8	11	22	33	24	37	24	29		=237
	90=38.0%				147=66.5%						
	negative				positive						
Meinicke	78=33.5%				156=66.5%				?3		=237

There was no definite increase in the number of positive reactions in the cases of pulmonary tuberculosis with cavity formation. The incidences were close to the figures which were obtained when all the exudative cases in the pulmonary tuberculosis series were separated into their own group. In the individual cases, however, it frequently occurred that all the serological reactions were simultaneously negative in recent cases in which there was cavity formation but only slight changes in the surrounding parenchyma.

Serological Reactions in Cases with Pneumothorax and Surgical Cases

I have also examined separately the patients with pulmonary tuberculosis who currently were undergoing pneumothorax treatment, and another group of patients who had been treated by a major surgical operation, such as pneumolysis, thoracoplasty, lobectomy or pneumectomy. No old post-surgical cases in which more than 2 years had elapsed since the operative treatment were included among the latter.

Table 17 shows the reactions in the sera of patients receiving collapse therapy. These patients are best comparable with cases

TABLE 17

RESULTS OF SEROLOGICAL TESTS IN CASES WITH PNEUMOTHORAX

Test	Titres									Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥1:256	
Hemagglutination	5	4	8	11	12	6	13			=59
	28=47.5%				31=52.5%					
Hemolysis	10	2	9	3	7	7	9	3	9	=59
	24=40.7%				35=59.3%					
Meinicke	negative				positive					
	25=42.4%				34=57.6%					=59

of unilateral exudative pulmonary tuberculosis. A comparison with the cases in the latter group revealed no decrease in the number of positive serological reactions because of the collapse therapy.

TABLE 18

RESULTS OF SEROLOGICAL TESTS IN SURGICAL CASES OF PULMONARY TUBERCULOSIS

Test	Titres									Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥1:256	
Hemagglutination	2	1	5	8	7	4	11			=38
	16=42.1%				22=57.9%					
Hemolysis	5	3	3	1	7	5	4	5	5	=38
	12=31.6%				26=68.4%					
Meinicke	negative				positive					
	17=41.5%				21=58.5%					=38

In the cases which had been treated operatively the process had usually been of the severe exudative type, and a comparison with these cases in the total series revealed no essential difference in the serological reactions of the two groups. It appears that the positive reactions persist for a long time post-operatively. Gernez-Rieux and Tacquet (108) followed up 9 surgical cases of pulmonary tuberculosis for about 2 years. In 6 cases recovery was good, but in only 4 of these a gradual decline in the titre set in, and in 1 case only it fell below 1: 8 during the time of observation. These workers, also, observed no effect by the pneumothorax on the reaction titres.

Relationship of Chemotherapy to Results of Serological Tests

During the present age of chemotherapy it is difficult to find patients with pulmonary tuberculosis who have not received this treatment. Thus among the 520 cases of pulmonary tuberculosis I found only 40 patients who according to the case history had not been given antituberculotic chemotherapy during the 4 months immediately preceding the drawing of the serological sample.

TABLE 19

RESULTS OF SEROLOGICAL TESTS IN CASES OF PULMONARY TUBERCULOSIS NOT TREATED BY CHEMOTHERAPY

Test	Titres									Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥1:256	
Hemagglutination	2	2	8	7	8	6	7			=40
	19=47.5%				21=52.5%					
Hemolysis	10	1	2	7	2	9	6	2	1	=40
	20=50.0%				20=50.0%					
Meinicke	negative				positive					
	11=37.5%				29=72.5%					=40

Table 19 shows the results of the serological tests in the cases which had not been treated by chemotherapy. A comparison with the entire case series reveals no clear difference in the reactions. The Meinicke test only gave, at 72.5 per cent, a higher incidence of positive reactions than the total series, but this group is too small to allow us to consider this difference a definite one.

INACTIVE CASES OF PULMONARY TUBERCULOSIS IN ADULTS

The following criteria were observed in the selection of the 107 cases in this series: The disease had a history of several years' duration, the sedimentation rate had been low for some time, no tubercle bacilli were present in the sputum, and there had been no development in the roentgenological changes. This series also includes those cases revealed by x-ray examination in which extensive calcifications were present in the lungs, but not cases with a calcified primary complex or hilar calcification only. Table 20 shows the results of the serological tests in this series.

TABLE 20

RESULTS OF SEROLOGICAL TESTS IN INACTIVE CASES OF PULMONARY TUBERCULOSIS

Test	Titres									Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≧1:256	
Hemagglutination	13	16	20	24	23	7	4			=107
	73=68.2%				34=31.8%					
Hemolysis	43	12	17	7	14	12	2	—	—	=107
	79=73.8%				28=26.2%					
Meinicke	negative				positive			? 1		=107
	82=77.4%				24=22.6%					

In both the hemagglutination and hemolysis tests the incidence of positive reactions in the inactive cases of pulmonary tuberculosis was slightly higher than in the series of non-tuberculous diseases (table 9) but definitely higher than in the series of healthy adults (table 8). The Meinicke test, on the other hand, had a lower incidence of positive reactions in this series than in non-tuberculous diseases but a definitely higher incidence than in healthy adults. Gernez-Rieux and Tacquet (108) in carrying out the hemagglutination test in 190 inactive cases of tuberculosis and the hemolysis test in 64 cases, obtained positive reactions in 85 cases (45 per cent) and 27 cases (42 per cent), respectively. My incidences of positive reactions were thus somewhat lower than those obtained by Gernez-Rieux and Tacquet.

Since it is the prevalent opinion that the serological reactions are considerably more frequently negative in extrapulmonary and childhood tuberculosis than in the pulmonary type, a separate series has been compiled of these cases. The results of the serological tests in extrapulmonary tuberculosis in adults are shown in table 21.

TABLE 21

RESULTS OF SEROLOGICAL TESTS IN CASES OF EXTRAPULMONARY TUBERCULOSIS

Form of Tuberculosis	No. of Cases		Titres									Meinicke		
			<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≥1:256	+	—	?
Tuberculous Pleural Empyema	3	Aggl.	—	—	—	—	1	—	2			3	—	—
		Hemol.	—	—	—	—	1	—	1	—	1			
Tuberculous Exudative Pleuritis	35	Aggl.	8	2	10	7	3	1	4			6	29	—
		Hemol.	14	8	3	4	1	2	1	—	2			
Tuberculous Meningitis	3	Aggl.	—	—	1	—	1	1	—			1	2	—
		Hemol.	1	—	1	—	—	—	—	1	—			
Bone and Joint Tuberculosis	7	Aggl.	—	1	2	1	1	2	—			6	—	1
		Hemol.	1	—	2	—	—	2	2	—	—			
Urogenital Tuberculosis	8	Aggl.	3	—	—	2	1	1	1			—	7	1
		Hemol.	4	—	1	1	—	1	—	—	1			
Tuberculosis of Lymph Nodes	5	Aggl.	2	—	—	2	—	1	—			3	2	—
		Hemol.	3	—	—	—	—	1	—	1	—			
Total	61	Aggl.	13	3	13	12	7	6	7			19	40	2
			41=67.2%				20=32.8%					32.2%	67.8%	
		Hemol.	23	8	7	5	2	6	4	2	4			
			43=70.5%				18=29.5%							

This series is relatively small and only contains 61 cases but it nevertheless indicates that the incidence of positive reactions is definitely lower in extrapulmonary than in pulmonary tuberculosis. Positive reactions were obtained in the Meinicke test in 19 cases out of 59 (32.2 per cent), in the hemagglutination test in 20 cases out of 61 (32.8 per cent) and in the hemolysis test in 18 cases out of 61 (29.5 per cent). The number of positive reactions was reduced

especially by the group of 35 cases of exsudative pleuritis, in 29 of which the Meinicke test was negative, in 27 the hemagglutination test, and in 29 the hemolysis test. In a series of extrapulmonary tuberculosis studied by Gernez-Rieux and Tacquet (108) a positive hemagglutination reaction was obtained in 67 cases out of 102 cases (66.4 per cent) and a positive hemolysis reaction in 29 out of 44 cases (65.9 per cent).

CHILDREN'S SERIES

Serological Reactions in Healthy Children and in Children with Non-Tuberculous Diseases

The healthy children in this series were either previously tuberculin-positive or were rendered tuberculin-positive by Calmette vaccination. As stated above, Gernez-Rieux and Tacquet (108) have demonstrated that BCG vaccination raises the titre in serological tests to the positive side for a short time. For this reason I have included no cases in which less than 1 year had elapsed since performance of the Calmette vaccination. The non-tuberculous diseases are not shown separately, since the two groups would be too small. The serological results for this series are shown in table 22.

TABLE 22

RESULTS OF SEROLOGICAL TESTS WITH SERA OF HEALTHY CHILDREN AND OF CHILDREN WITH NON-TUBERCULOUS DISEASES

Test	Titres									Total
	<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≧1:256	
Hemagglutination	41	29	42	44	12	4	—			=172
	156=90.7%				16=9.3%					
Hemolysis	86	11	26	21	19	5	4	—	—	=172
	144=83.7%				28=16.3%					
Meinicke	negative				positive					
	143=84.6%				26=15.4%		?3			=172

The incidence of positive reactions was about 10 per cent lower than that obtained in non-tuberculous diseases in adults. However, it was as high as in the series of healthy adult subjects except in the Meinicke reacton, in which the incidence of positive reactions

TABLE 23

RESULTS OF SEROLOGICAL TESTS IN CHILDHOOD TUBERCULOSIS

Form of Tuberculosis	No. of Cases		Titres									Meinicke		
			<1:2	1:2	1:4	1:8	1:16	1:32	1:64	1:128	≧1:256	+	—	?
Pulmonary tuberculosis.....	5	Aggl.	1	1	1	—	—	1	1			3	2	—
		Hemol.	3	—	—	1	—	1	—	—	—			
Pulmonary and bone and joint tuberculosis	4	Aggl.	—	—	—	1	1	—	2			3	1	—
		Hemol.	—	1	1	—	1	—	—	—	1			
Exudative pleuritis	2	Aggl.	1	1	—	—	—	—	—			—	2	—
		Hemol.	2	—	—	—	—	—	—	—	—			
Miliary tuberculosis	2	Aggl.	—	1	1	—	—	—	—				1	1
		Hemol.	2	—	—	—	—	—	—	—	—			
Tuberculous meningitis	12	Aggl.	1	—	2	2	2	3	2			4	7	1
		Hemol.	3	1	1	2	2	1	1	—	1			
Tuberculous pericarditis	1	Aggl.	—	—	1	—	—	—	—			1	—	—
		Hemol.	1	—	—	—	—	—	—	—	—			
Renal tuberculosis	3	Aggl.	—	1	—	2	—	—	—			—	3	—
		Hemol.	1	1	1	—	—	—	—	—	—			
Tuberculosis of bones and joints	99	Aggl.	13	13	18	21	20	10	4			43	55	1
		Hemol.	18	15	9	26	18	7	2	3	1			
Tuberculosis of lymph nodes	8	Aggl.	3	—	2	2	1	—	—			2	6	—
		Hemol.	6	—	—	—	2	—	—	—	—			
Tuberculosis of eye	1	Aggl.	—	—	1	—	—	—	—			—	1	—
		Hemol.	1	—	—	—	—	—	—	—	—			
Total	137	Aggl.	19	17	26	28	24	14	9			56	78	3
			90 = 65.7 %				47 = 34.3 %					41.7 %	58.3 %	
		Hemol.	37	18	12	29	23	9	3	3	3			
			96 = 70.0 %				41 = 30.0 %							

was about 10 per cent higher than in healthy adults. Andersson and Platou (88) and Gernez-Rieux and Tacquet (108) are of the opinion that children usually give low titres. The first mentioned regarded a titre of 1: 4 as positive and the latter a titre of 1: 8. In view of the results in my control series a titre below 1: 16 could not be considered positive in Finland.

Childhood Tuberculosis

The childhood tuberculosis series comprised a total of 137 cases. Children's sera appear to have titres that are lower than those obtained in my series of adult tuberculotics. This is seen very clearly in the group of 99 cases of tuberculosis of bones and joints, in table 23.

The results which I was able to obtain are definitely poorer than those of Gernez-Rieux and Tacquet (108) for in a series of childhood tuberculosis of various types they had positive reactions in 71 out of 77 cases (92.2 per cent) in the hemagglutination test and in 32 out of 45 cases (71.1 per cent) in the hemolysis test. The antigen used was I.P. 48 and titres of 1: 8 and higher were interpreted as positive. If this titre were considered positive in my series, the incidence of positive reactions would be 54.7 per cent in the hemagglutination test and 51.1 per cent in the hemolysis test.

EVALUATION OF THE BORDERLINE TITRE

In table 24 I have calculated the percentages of positive reactions in the hemagglutination and hemolysis tests when the titres of 1: 8, 1: 16 and 1: 32 are regarded as the borderline for positive reactions. The series of extrapulmonary tuberculosis and all the children's series have been omitted from this table.

In the hemagglutination test the results are more dependent on the selection of the borderline titer than in the hemolysis test. For instance, if the borderline is raised to 1: 32, the incidence of positive reactions in the hemagglutination test will decline by 8 per cent in the series of healthy adults but by 21 per cent in the pulmonary tuberculosis series. In the hemolysis test the decline is 5.5 per cent and 13.3 per cent, respectively. In general, a relatively large proportion of the results in the hemagglutination test are near

TABLE 24

PERCENTAGE OF POSITIVE REACTIONS IN HEMAGGLUTINATION AND HEMOLYSIS TESTS, DIFFERENT BORDERLINES

Test	Lowest Positive Titre	Healthy Subjects	Non-Tuberculous Diseases	Inactive Pulmonary Tb	Active Pulmonary Tb
Hemagglutination	1: 8	29.5%	42.6%	54.2%	66.9%
	1: 16	11.1%	21.3%	31.8%	49.8%
	1: 32	3.0%	5.8%	10.3%	28.8%
Hemolysis....	1: 8	20.7%	36.6%	32.7%	65.8%
	1: 16	15.5%	25.5%	26.2%	54.6%
	1: 32	10.0%	15.3%	13.1%	41.3%

the titres which may be considered decisive, whereas in the hemolysis test the results are spread over a wider range.

The selection of titre 1: 16 as the borderline for positive reactions appears to be well founded in the hemagglutination test, for the most distinct difference is then obtained between the incidence in the active pulmonary tuberculosis series and the other test series. If a titre of 1: 8 is taken as the borderline, the pulmonary tuberculosis series will show a difference of 37.4 per cent from the healthy adult series, 24.3 per cent from the series of non-tuberculous diseases, and 12.7 per cent from the inactive pulmonary tuberculosis series. With the borderline at 1: 16 the differences are 38.7 per cent, 28.5 per cent and 18.0 per cent, respectively, and at 1: 32 they are 25.8 per cent, 23.0 per cent and 18.5 per cent.

In the hemolysis reaction a borderline of 1: 8 would seem to be more favorable, for the difference between the incidences in active pulmonary tuberculosis and in the other series is greatest at this level, *i.e.* 45.1 per cent when compared with the series of healthy subjects, 29.2 per cent when compared with non-tuberculous diseases, and 33.1 per cent compared with inactive pulmonary tuberculosis. At a borderline of 1: 16 these differences would be 39.1 per cent, 29.1 per cent and 28.4 per cent, respectively, and at 1: 32 they would be 31.3 per cent, 26.0 per cent and 28.2 per cent. Since the difference in the hemolysis test is a very small one whether the borderline is at 1: 8 or at 1: 16, I have for the sake of uniformity selected the latter titre as the lowest positive value.

SUMMARY OF SEROLOGICAL TEST RESULTS

It was one of the purposes of this investigation to study which combination of the three serological tests used by me would form the best aid in the clinical diagnosis of tuberculosis. To fill this purpose it is required that such a combination will give positive reactions in a comparatively large number of tuberculous cases but a minimum of positive reactions in the control cases. Since in the studies described above the number of positive and negative reactions in the three tests lie fairly close to each other, it seemed probable that a suitable combination would hardly be obtainable. I have, however, classified the entire series in different combinations of positive and negative reactions obtained in the three tests, as shown in table 25. All cases of syphilis and those cases in which the Meinicke test showed a positive reaction in the control tube have been excluded from this table.

TABLE 25

DISTRIBUTION OF ALL CASES ACCORDING TO RESULTS OF SEROLOGICAL TESTS

Meinicke	+	+	+	+	—	—	—	—	Total
Hemagglutination	+	+	—	—	+	+	—	—	No. of
Hemolysis	+	—	+	—	+	—	+	—	Cases
Healthy subjects	1	3	2	17	13	20	56	259	=371
Pregnancy and nontuberculous diseases	28	18	27	47	15	15	26	227	=403
Inactive pulmonary tuberculosis	8	4	1	11	10	12	7	53	=106
Active tuberculosis	165	53	59	107	64	40	50	168	=706
Total	202	78	89	182	102	87	139	707	1586

It will be observed that a positive reaction occurred in all the three tests in 23 ± 1.6 per cent of cases of tuberculosis in the active stage and in 4.2 ± 0.8 per cent of the control series. All the tests were negative in active tuberculosis in 24 ± 1.6 per cent and in the control series in 56 ± 2.5 per cent.

Table 26 shows the percentages of positive and negative reactions in each series of cases, distributed according to positive reactions in one test and in two or three tests, and according to negative reactions in at least two tests or in all three tests. The percentage of statistical deviation of the results is also given.

TABLE 26

PERCENTAGE DISTRIBUTION OF THE CASE SERIES ACCORDING TO SEROLOGICAL TEST RESULTS AND ITS DEVIATION

	Per cent of Cases Positive in at least 2 Tests	Per cent of Cases Negative in at least 2 Tests	Per cent of deviation of results	Total No. of Cases
Healthy subjects ..	5	95	1.1	371
Pregnancy and non-tuberculous diseases	22	78	2.0	403
Inactive pulmonary tuberculosis	22	78	4.0	106
Active tuberculosis	48	52	1.8	706
	Per cent of Cases Positive in at least 1 Test	Per cent of Cases Negative in all 3 Tests	Per cent of Deviation of Results	Total No. of Cases
Healthy subjects ..	30	70	2.4	371
Pregnancy and non-tuberculous diseases	44	56	2.5	403
Inactive pulmonary tuberculosis	50	50	4.9	106
Active tuberculosis	76	24	1.6	706

Two or more tests showed positive reactions in 5 ± 1.1 per cent in the series of healthy subjects but as much as 22 ± 2.0 per cent in non-tuberculous diseases and pregnancy. In inactive pulmonary tuberculosis the percentage was also 22 per cent, but the statistical deviation was greater because of the smaller number of cases in the series. In active tuberculosis, 48 ± 1.8 per cent of cases were positive in two or three tests. The difference between the active tuberculosis series and the control series is significant but not very great in all the combinations of tests.

ABSORPTION TESTS FOR REMOVAL OF POSSIBLE NON-SPECIFIC REACTIONS

Principles of the Test: Agglutinins produced by the disease can be removed from the serum by absorption of the latter with a sufficient amount of the bacterium causing the disease.

Technic and Results: Since absorption with sheep red cells

prior to performance of the hemagglutination and hemolysis tests may possibly be inadequate, I carried out this step in some cases a number of times. This was considered necessary also because of the observation made by Sievers *et al.* (144) that positive hemagglutination reactions disappear after repeated absorption with sheep red cells if the rise in the titre is not due to tuberculosis. I therefore examined the sera of 47 patients with tuberculosis and of 59 control subjects by the hemagglutination and hemolysis tests after the routine absorption of the sheep red cells and repetition of the absorption 6 times. The results of the tests in these cases are listed in table 27.

TABLE 27

RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS BEFORE AND AFTER ABSORPTION OF SERA WITH SHEEP RED CELLS SIX TIMES

Test	No. of Case	Before Absorption		After Absorption		Changed to Posit.	Titre		
		Posit.	Negat.	Posit.	Negat.		Decr.	Incr.	Unchanged
Hemagglutination									
Tuberculotics	47	38	9	27	20	1	30	7	10
Controls	59	26	33	15	44	1	26	3	30
Hemolysis									
Tuberculotics	47	36	11	27	20	3	24	16	7
Controls	59	37	22	25	34	5	31	14	14

The reaction was considered positive if the titre was 1: 16 or higher. After absorption 6 times about one-third of the sera became negative, having a titre of 1: 8 or lower, but this occurred to an equal extent in the control series and in the tuberculosis series. A slight fall in the titre occurred almost regularly in all cases, which was only natural since the serum becomes diluted in connection with absorption. It thus seems probable that repeated absorption with sheep red cells will not remove possible non-specific reactions.

I also absorbed a number of sera with dried powdered *Staphylococcus aureus-albus* and some sera with dried powdered tubercle bacilli. Staphylococci were employed since this is perhaps the most common bacterium in man and in the case of a non-specific reaction caused by this bacterium the reactions would presumable become negative in at least a part of the

cases after this absorption procedure. Absorption with tubercle bacilli should make the reactions negative if a specific serological tuberculosis reaction is involved. In similarity with Maillard and Gagliardo (130) I used 50 mg of powdered bacteria per ccm of serum diluted 1: 2 with physiological saline solution. The *Staphylococcus aureus-albus* powder was prepared by culture of the strains on the usual agar for 3 days, after which the bacteria were suspended in physiological saline solution, killed by keeping at +120°C for 40 min. and then dried in the incubator. The tubercle bacillus powder was prepared from strain $H_{37}Rv$, which had been grown for 6 weeks on Sauton culture medium.

The absorption with staphylococci was carried out in the case of 100 tuberculous sera and 111 control sera. Table 28 shows the changes to negative in the sera which were positive before absorption and the movement of the titres for all the sera absorbed with the dried powdered staphylococcal mixture.

TABLE 28

RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS BEFORE AND AFTER ABSORPTION OF SERA WITH STAPHYLOCOCCAL MIXTURE

Test	No. of Case	Before Absorption		After Absorption		Changed to Pos.	Titre		
		Posit.	Negat.	Posit.	Negat.		Decr.	Incr.	Unchanged
Hemagglutination									
Tuberculotics	100	50	50	24	76	1	70	6	24
Controls	111	28	83	5	106	3	76	6	29
Hemolysis									
Tuberculotics	100	61	39	23	77	2	77	6	17
Controls	111	36	75	13	98	6	56	15	40

In the hemagglutination test in particular the absorption with staphylococci appeared to shift the reaction to the negative side more frequently in the control series than in the tuberculosis series. However, this difference may be only a seeming one, for in control cases the titres usually were lower than in tuberculotics and more frequently grouped around the borderline of positive reactions, so that a fall in the titre even by one dilution brought the reaction to the negative side. On the other hand, a comparison of the decline in the titre values after absorption shows that this was approximately the same in both groups. In the hemolysis test,

also, the control series showed more frequent changes to negative than the tuberculotic series, but in this test the titre often was elevated following absorption with staphylococci, as also had been the case after repeated absorption with sheep cells.

The sera of 41 tuberculous persons and 32 control subjects were absorbed with killed and dried tubercle bacilli. In the hemagglutination test following absorption the titre was below 1: 2 in 35 of the former and 28 of the latter, and in the hemolysis test in 37 and 27, respectively. In only one case in the control series the hemolysis test remained positive after the absorption with tubercle bacilli. The results will be seen in figs. 6—9.

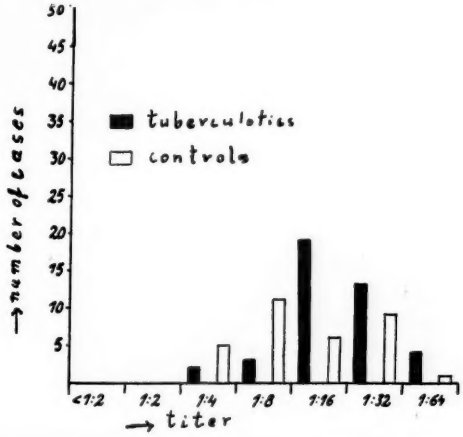

Fig. 6. — Hemagglutination titres before absorption of sera with tubercle bacilli.

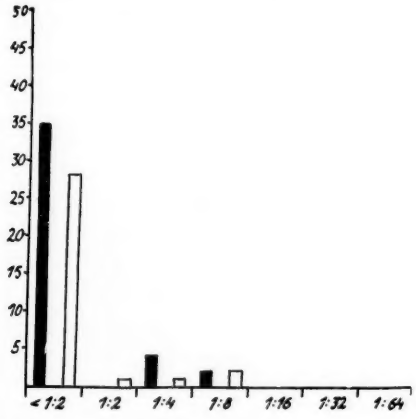

Fig. 7. — Hemagglutination titres after absorption of sera with tubercle bacilli.

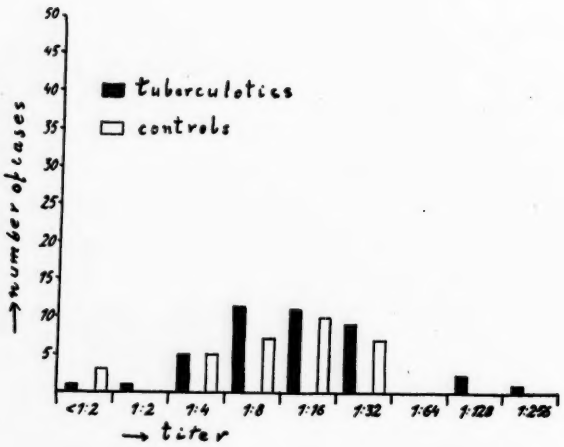

Fig. 8. — Hemolysis titres before absorption of sera with tubercle bacilli.

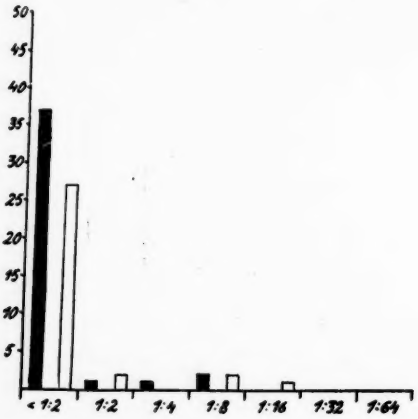

Fig. 9. — Hemolysis titres after absorption of sera with tubercle bacilli.

Following the absorption with staphylococci the titre fell below 1: 16 in about one-half of the cases of tuberculosis, whereas after the absorption with tubercle bacilli all tuberculotic sera became negative. It would thus seem probable that the titres obtained in these cases were infact due to the tuberculosis infection. In the control series the absorption with staphylococci made the hemagglutination test negative in about 80 per cent of the cases and the hemolysis test in about 65 per cent of the cases. The absorption with tubercle bacilli effected this change in a still greater number of cases but the difference was not very marked.

EXAMINATION OF THE ANTIGEN BY MACRO-ELECTROPHORESIS

I have made an attempt to find out by means of electrophoresis which part of the antigen contains the active substance. For this purpose the antigen was fractionated by the macro-electrophoresis method.

In the procedure, used 1 gm of lyophilised antigen was dissolved in 100 ccm of barbituric acid-sodium diethylbarbiturate buffer, ionic strength 0.1 and pH 8.6. The solution was dialysed against the same buffer at +4°C until balance was obtained, determined by measuring the conductivity of the solution and the buffer. The electrophoresis run was made in the Tiselius apparatus at +2°C with a potential gradient of 9V/cm. After 3½ hrs. the run was stopped and the fluid columns in the ascending limb (positive pole) and the descending limb (negative pole) of the U tube were cut off in the middle in the usual way. The components with the highest speed were then located in the upper part of the ascending limb and those migrating slowest were in the upper part of the descending limb.

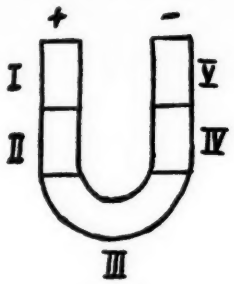

Fig. 10. — Position of Fractions I—V after completed electrophoretic run.

The result of the run be seen in fig. 10.

The nitrogen content of the various components, determined by the biuret method, was as follows:

Fraction	I: Upper part of ascending limb	0.4	per cent N
»	II: Lower » » » »	0.5	» » »
»	III: Base	0.5	» » »
»	IV: Lower part of descending limb	0.3	» » »
»	V: Upper » » » »	0.1	» » »

Sheep red cells were sensitised in the usual manner with the 5 fractions obtained by electrophoresis, using a volume of solution corresponding to an antigen concentration of 10 mg per 0.1 ccm of cells. The titrations were made with 5 different sera and the titres

were compared with those obtained at the same time with the original antigen. The fractionation and the serum titrations were performed twice and the results obtained were in agreement with each other. From table 29 will be seen that Fractions I—IV gave approximately the same titres as the original antigen. On the other hand, fraction V, which had the lowest nitrogen concentration, was completely inactive.

TABLE 29

RESULTS OF HEMAGGLUTINATION AND HEMOLYSIS TESTS USING SHEEP RED CELLS SENSITISED WITH THE ORIGINAL ANTIGEN AND FIVE MACROELECTROPHORETICALLY SEPARATED FRACTIONS

Test	Serum No.	Antigen Used for Sensitisation					
		Original Antigen	I	II	III	IV	V
Hemagglutination ..	1	1: 16	1: 8	1: 16	1: 16	1: 8	0
	2	1: 16	1: 8	1: 16	1: 8	1: 8	0
	3	1: 4	1: 2	1: 2	1: 2	0	0
	4	1: 4	1: 2	1: 4	1: 8	1: 8	0
	5	1: 16	1: 16	1: 16	1: 32	1: 16	0
Hemolysis.....	1	1: 8	1: 8	1: 16	1: 8	1: 16	0
	2	1: 2	1: 4	1: 8	1: 2	1: 2	0
	3	0	0	0	0	0	0
	4	1: 2	1: 2	1: 4	1: 2	0	0
	5	1: 4	1: 8	1: 2	0	1: 8	0

DISCUSSION

In reviewing the results obtained in this investigation with the Meinicke, hemagglutination and hemolysis tests for tuberculosis, two questions stand out. Firstly, what is the reason for the negative serological reactions in a considerable proportion of tuberculous processes which clearly are in the active phase? Secondly, why do clinically healthy persons and patients with non-tuberculous diseases frequently give positive reactions in the serological tests for tuberculosis?

Several investigators have studied these problems in the past. As stated by Meinicke (54), the serological reactions for tuberculosis are based on the demonstration of an excess of antibody, and if the disease process is in a very active phase, associated with tissue decomposition and large amounts of tubercle bacilli in the body the latter completely bind the antibodies produced by the organism, leaving no excess. In support of this opinion he referred to the negative reactions frequently obtained in the exudative type of pulmonary tuberculosis and when tuberculosis nears a fatal termination; in both conditions the number of bacilli in the body is known to be very large. However, in my series this phenomenon was not seen and, in fact, the highest incidence of positive reactions occurred in exudative pulmonary tuberculosis. Similarly, the 17 cases which ended fatally within 2 months from the time of drawing the sample gave positive Meinicke and hemagglutination reactions in 13 cases and positive hemolysis reactions in 9 cases. Thus the incidence of positive reactions in fatal cases was 76.4 per cent in the Meinicke and hemagglutination tests and 52.9 per cent in the hemolysis test, while in the entire series of cases of pulmonary tuberculosis the Meinicke test was positive in 58.9 per cent, the hemagglutination test in 49.8 per cent and the hemolysis test in 54.6 per cent.

It may also be presumed that sera that give a negative reaction may contain substances that inhibit the tuberculosis reactions. This possibility was studied by Gernez-Rieux and Tacquet (108), but on adding to positive serum negatively reacting serum from a tuberculotic patient they found a weakening of the positive reaction in exceptional cases only. Using in the hemagglutination and hemolysis tests Coombs's technic, which is employed in the examination of Rh antibodies, these investigators as well as Meynell (131) studied whether the blood of otherwise negatively reacting patients with tuberculosis possibly contains incomplete antibodies which give a positive reaction by this technique only. However, the results were negative in both the hemagglutination and the hemolysis reactions. Gernez-Rieux and Tacquet (108) also failed to observe the so-called »Zone» phenomenon, in which there is a stronger reaction in some serum dilutions. In a few cases I was able to observe this phenomenon to a small degree in the hemolysis reaction, but in my opinion it is of no practical significance in the evaluation of the results.

According to reports in the literature and my own investigations, the most important factor in the occurrence of negative reactions in tuberculosis is probably the antigen itself. Thus greatly varying results were obtained by Gernez-Rieux and Tacquet in employing as antigen the phenol-water extract of tubercle bacilli, prepared according to the Middlebrook-Dubos method described above, or I.P. 48 or its polysaccharide fraction from the Pasteur Institute. Grabar *et al.* (111) have demonstrated that in some cases the red cells sensitised with the I.P. 48 protein fraction or PPD after treatment with tannic acid reacted positively with sera which gave a positive reaction with sensitised cells that had not been pretreated with tannic acid. Middlebrook (133) suggested that the active substance in the hemolysis reaction is a component in the antigen different from that in the hemagglutination reaction. Occasionally, it is true, such marked differences are seen in the titres obtained for the same sera in the hemagglutination and hemolysis tests that there seems to be reason to suspect the action of different components. Reactions of this kind, in which the difference in the titres was 16-fold or greater, were seen in 79 of the 825 sera in my series of tuberculous cases and in 42 of the 823 sera in the control series.

Chemically, three groups of substances are present in the tubercle bacillus, *i.e.*, lipids, proteins and polysaccharides. The antigen which is active in the hemagglutination and hemolysis reactions contains only the latter two, but the Meinicke antigen has all the three groups. In my investigation the Meinicke test yielded the best results. It would thus seem that the exclusion of lipids from the antigen leads to a deterioration of the results. It furthermore seems that tuberculosis induces a highly complicated antibody formation in the organism and apparently there occur antibodies against different components of the tubercle bacilli in different cases. The amounts of antibodies in the blood are probably small, for the titre levels obtained were usually low, and in some cases they apparently are not present in demonstrable amounts.

The occurrence of positive reactions in clinically healthy individuals may also be due to a number of causes. It seems probable that antibodies from healed tuberculosis persist in the blood for a long time. The 107 inactive cases of pulmonary tuberculosis in my series had positive reactions in the Meinicke test in 22.6 per cent, in the hemagglutination test in 31.8 per cent, and in the hemolysis test in 26.2 per cent. These incidences are higher than in the series of non-tuberculous diseases and healthy individuals except in the non-tuberculous series in the Meinicke test. Sievers *et al.* (144) demonstrated that persons who had been in contact with tuberculotics gave a larger number of positive reactions than other healthy persons. Gaby *et al.* (101) also observed that the laboratory personnel reacted positively more frequently than was the case normally. Haahti and Raitio (112), again, reported that umbilical cord blood gave a positive reaction if the mother had a high hemagglutination titre. The tuberculin test may occasionally cause a positive reaction to appear. Thus Gernez-Rieux and Tacquet after injection of increasing doses of tuberculin into 6 tuberculin-negative persons who gave negative serological reactions found a positive hemagglutination reaction in 1 case. BCG vaccination, also, will give a positive titre in 50 per cent of the subjects according to Gernez-Rieux and Tacquet, but this high titre does not persist for more than 1 year at the most.

Since tuberculosis may sometimes heal so completely that no post-tuberculous changes can be recognised clinically, and since in Finland tuberculosis is so prevalent that healthy persons may

readily come into contact with diseased persons and thus contract subclinical tuberculosis, the possibility cannot be excluded that some of the positive reactions in my series of healthy individuals should be ascribed to this cause. Calmette vaccination, on the other hand, has thus far been performed in Finland almost exclusively on the new-born and my material hardly includes cases in which this would be the possible cause of positive reactions. The greatest difficulty is encountered in explaining the occurrence of the frequent positive reactions in tuberculin-negative persons, for the tuberculin test is generally considered so sensitive that it seldom gives a negative reaction in cases with a history of tuberculosis.

In animal experiments, Fisher (100) obtained positive reactions in the hemagglutination and hemolysis tests with bacilli of the *Mycobacterium* species. Gaby *et al.* (102) inhibited these reactions by means of tuberculins prepared from apathogenic *Mycobacteria* of various types. It has been demonstrated earlier that the Meinicke reaction is as frequently positive in leprosy as in tuberculosis, and Levine (123) showed that the same is true with regard to the hemagglutination reaction. Meinicke (59) considered the diphtheria bacillus to be so closely related to the tubercle bacillus that it may cause positive reactions in serological tests for tuberculosis. *Mycobacteria* which are pathogenic for animals are not usually pathogenic for man. Apathogenic species are occasionally found in human subjects but since they usually do not produce a diseased condition but exist as saprophytes they probably induce no antibody formation. Leprosy is practically extinct in Finland. During the Second World War, the incidence of diphtheria was very high in Finland but it has declined greatly during the recent years. In my series there is only 1 case of diphtheria, and it gave positive reactions in all the three tests. However, in view of the few cases that have occurred in Finland in the past few years, it does not seem probable that this disease would have caused false positive reactions in my series. Therefore the effect of bacteria mentioned above, on the reactions in my control series must be regarded as very small or non-existent.

Eickhoff (19) made the observation that serological tests for tuberculosis frequently give positive reactions in acute infectious diseases and advanced the opinion that non-tuberculous infections may raise again the amount of antibody against tuberculosis. The

cases of scarlatina in my series showed positive Meinicke reactions in 40.4 per cent, hemagglutination reactions in 34.5 per cent and hemolysis reactions in 25.9 per cent. These figures are slightly higher than those for non-tuberculous diseases in the entire material. The same observation was made with regard to pregnancy, as in this series the Meinicke test was positive in 47.6 per cent, the hemagglutination test in 33.3 per cent and the hemolysis test in 53.3 per cent. The difference was thus still greater between the pregnancy series and the series of non-tuberculous diseases. It is difficult to state whether we have here the stimulation of antibody formation suggested by Eickhoff or a true non-specific reaction.

Although, as seen from the foregoing, there are various possibilities for explanation of the occurrence of negative reactions in tuberculosis and positive reactions in non-tuberculous diseases and healthy persons, I am of the opinion that the results obtained in my investigation are evidence that the serological tests which I have studied are specific for tuberculosis infection. The following observations support this opinion:

1) The serological tests studied by me gave the highest incidence of positive reactions in cases of tuberculosis. Positive reactions were obtained in the different tests in 50—60 per cent of cases of pulmonary tuberculosis, in 30—35 per cent of extrapulmonary tuberculosis, and in 30—40 per cent of childhood tuberculosis. In non-tuberculous diseases, the reactions were positive in 20—30 per cent and in healthy subjects in 5—15 per cent.

2) A correlation between the clinical picture of the disease and the positive reactions was evident. Thus the extensive processes of exudative and exudative-productive types of pulmonary tuberculosis gave the highest number of positive reactions; this was also true of cases with a history of pulmonary tuberculosis of several years' duration and those with a high sedimentation rate.

3) Absorption of the sera with a mixture of dried powdered staphylococci produced no marked fall in the titre levels, and at least a part of the slight decline may be ascribed to dilution of the serum during the absorption procedure. On the other hand, following the absorption of the sera with dried tubercle bacilli the positive reactions disappeared completely from the sera of almost all tuberculotics and control subjects.

4) Statistically there was a definite difference between the incidence of positive reactions in healthy individuals, pregnant females and patients with non-tuberculous diseases and the incidence in patients with active tuberculosis. Thus all the three tests were negative in the series of healthy persons in 70±2.4 per cent, in pregnancy and non-tuberculous diseases in 56±2.5 per cent, and in active tuberculosis 24±1.6 per cent. In the same series, at least two tests were [illegible] in 5±1.1 per cent, 22±2.0 per cent, and 48±1.8 per cent [illegible]ively.

5) In animal tests none of the [illegible]hy guinea pigs gave a positive reaction, whereas 51.4 per c[illegible] of the guinea pigs infected with tubercle bacilli were positive in the hemagglutination test and 71.4 per cent in the hemolysis test.

SUMMARY

In these studies on the diagnostic value of certain modern serological tests for tuberculosis, three test methods were employed. The Meinicke test was performed according to the »Kuppen»technique, with main importance on the second reading of reactions. In the Middlebrook-Dubos hemagglutination test and in Middlebrook's hemolytic modification of this test the antigen was a lyophilised powder prepared by me from tuberculin formed by tubercle bacillus strain $H_{37}Rv$ grown on Sauton culture medium. The strength of the antigen was determined by parallel titrations with Lederle, Berna and PPD Copenhagen tuberculins. Titres of 1: 16 and higher were regarded as positive in the hemagglutination and hemolysis tests.

My case material comprised 718 cases of tuberculosis in the active phase, 107 cases in an inactive phase, and a control series of 271 healthy adults, 302 patients with non-tuberculous diseases, 33 syphilitics, 45 pregnant females and 172 children who were healthy or affected with non-tuberculous diseases.

In active pulmonary tuberculosis the highest incidence of positive reactions was obtained in the Meinicke test, in which 302 out of 513 cases (58.9 per cent) were positive. In the hemagglutination test there were 259 positive reactions among 520 cases (49.8 per cent) and in the hemolysis test 284 positives in 520 cases (54.6 per cent). Extrapulmonary tuberculosis in adults gave positive reactions in 19 out of 59 cases (32.2 per cent) in the Meinicke test, 20 out of 61 cases (32.8 per cent) in the hemagglutination test and 18 out of 61 cases (29.5 per cent) in the hemolysis test. In inactive pulmonary tuberculosis the Meinicke test was positive in 24 out of 106 cases (22.6 per cent), the hemagglutination test in 34 out of 107 cases (31.8 per cent) and the hemolysis test in 28 out of 107 cases (26.2 per cent). Positive reactions in childhood tuberculosis were obtained in 56 out of 134 cases (41.7 per cent) in the Meinicke

test, in 47 out of 137 cases (34.3 per cent) in the hemagglutination test, and in 41 out of 137 cases (30.0 per cent) in the hemolysis test.

In the series of healthy adults the Meinicke test was positive in 5.5 per cent, the hemagglutination test in 11.1 per cent, and the hemolysis test in 15.3 per cent. The corresponding incidences in non-tuberculous diseases and pregnancy were 31.5 per cent, 21.3 per cent and 25.5 per cent, respectively, and in healthy children and children with non-tuberculous diseases they were 15.4 per cent, 9.3 per cent and 16.3 per cent.

In relation to the clinical picture of pulmonary tuberculosis, the highest incidence of positive reactions occurred in cases of extensive exudative or exudative-productive processes in the lungs. Thus the Meinicke reaction was positive in bilateral exudative pulmonary tuberculosis in 72.2 per cent, the hemagglutination reaction in 58.4 per cent, and the hemolysis reaction in 60.9 per cent of cases. In cases with a history of pulmonary tuberculosis of 5 years' duration or longer the tests were positive in 78.5 per cent, 59.0 per cent and 62.3 per cent, respectively, and in cases with a sedimentation rate of 60 mm/l hr. and over the incidences were 72.7 per cent, 65.8 per cent and 55.3 per cent. Therapeutic measures of various kinds, such as chemotherapy and pneumothorax and other surgical procedures, did not appear to reduce the incidence of positive reactions.

No improvement was obtained in the specificity of the hemagglutination and hemolysis tests by repeated absorption of the sera with sheep red cells, for the titres were reduced in both the tuberculosis series and the control series through this procedure. Absorption with staphylococci, likewise, did not reduce the number of positive reactions in the control sera to any greater extent than in the tuberculous sera. On the other hand, absorption with tubercle bacilli changed nearly all the positive reactions to negative.

After macro-electrophoretic fractionation of the antigen the fraction with the lowest protein content did not appear to sensitise red cells for the tuberculosis reactions.

Negative reactions were obtained in all three serological tests in 70 ± 2.4 per cent of healthy subjects, in 56 ± 2.5 per cent of cases of non-tuberculous diseases and pregnancy, in 50 ± 4.9 per cent of cases of inactive pulmonary tuberculosis, and in 24 ± 1.6 per cent

of cases of active tuberculosis. At least two tests were positive in 5 ± 1.1 per cent of healthy individuals, 22 ± 2.0 per cent of adults with non-tuberculous diseases and pregnant females, 22 ± 4.0 per cent in inactive pulmonary tuberculosis, and 48 ± 1.8 per cent in active tuberculosis.

The three serological tests used in this investigation showed approximately the same incidence of positive reactions in tuberculosis. In the control series the results were also very similar in the three tests. On the basis of the results obtained in these two series, a combination of the Meinicke and the hemagglutination tests seems to be the most serviceable.

The clinical values of the serological tests for tuberculosis studied in this investigation is moderate, for the reactions become positive only after fairly extensive areas are involved and the disease is already recognisable by other clinical methods. Furthermore, positive reactions are frequently obtained in cases in which evidence of an active tuberculotic process is not found by clinical methods of examination.

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